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News

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Seed Treating News

from DU PONT

FAVORABLE "ARASAN" FIELD REPORTS CONTINUE THIS SUMMER

Additional information about practical farm tests in treating grass and legume seed with Du Pont "Arasan" seed disinfectant is being gathered this summer.

Many tests were put out this spring, and early reports show the same pattern of results found in 1951 spring and fall tests. Stand counts are now being made from every part of the northern United States on about the same crops as were tested last year. And, some new ones are under test this year, too.

Among the early reports are three alfalfa tests from New York, the poorest of which showed a 40% increase in stand from seed treated with "Arasan" over untreated seed.

Following up some of last year's tests is also uncovering important information. Better yields of hay have been reported from areas where treated seed was planted in 1951.

As soon as the majority of these tests are tabulated another report, Number 3, will be issued. But, for now, if you have not received a copy of No. 2, which reported 25 tests in southern states, planted last fall, write and ask for A-3642.

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CROP ROTATION IN RELATION TO THE GOLDEN NEMATODE POPULATION OF THE SOIL[1]

W. F. Mai and B. F. Lownsbery

In countries where large areas of land suitable for growing potatoes are heavily infested with the golden nematode, *Heterodera rostochiensis* Wollenweber, crop rotation is the chief control measure. According to observations and experiments of numerous workers the length of rotation necessary for profitable potato production increases with degree of infestation. When the infestation becomes older and the number of nematodes in the soil increases, 4, 5, or 6-yr. rotations are necessary for a profitable yield.

Compared with continuous potato production 5 3-yr. rotations resulted in 75 to 90 per cent reduction in numbers of full cysts in the soil (4). In rotations involving barley, oats, rye, wheat, clover, and alfalfa the numbers of full cysts in the soil were back to the original level following a crop of potatoes. Growing corn a single season brought about a 54 per cent reduction, and lupines a 16 per cent reduction in viable nematode population (3). On Long Island, following a single year's cultivation of cabbage, beans, mustard, rape, carrots, oats, or wheat, potato yields were no greater than when potatoes were grown continuously. Soil samples taken after the potato crop from plots in which rotation was practiced contained no fewer nematodes than samples from plots continuously cropped to potatoes (2). No significant differences were observed in the number of viable golden nematodes in the soil after the potato crop when flax, barley, oats, beets, field beans, wheat, chicory, peas, carrots, mustard, and corn were grown in 3-yr. rotations with potatoes (6). Since different crops were used in the same rotation it was not possible to compare their relative effectiveness in reducing the nematode population.

Experiments in which commercial crops were grown in 3-yr. rotations with potatoes under Long Island conditions to test their relative effects on the viable nematode population of the soil are reported here.

PROCEDURE.—Two rotation experiments were started in the spring of 1946. Plots 8 by 10 ft. were located in a potato field very lightly infested by the golden nematode. Six to 8 in. of heavily infested soil, obtained from several locations within a relatively small area, were added to each plot to raise the level of infestation uniformly. Twelve soil samples were taken from each plot to a depth of 6 to 8 in. by means of a soil auger. There were from 4 to 7 viable cysts per 10 gm. of air-dried soil. In order to minimize transfer of cysts between plots, 12-in. boards, sunk into the soil for 6 in. were placed around each plot.

Only 3-yr. rotations were included in these experiments because 2-yr. rotations have been shown to be ineffective and longer rotations were not considered practical for Long Island potato growers operating on high priced land.

In the first experiment green bean, *Phaseolus vulgaris* L., sugar corn (*Zea Mays* var. *saccharata* Bailey), perennial rye grass (*Lolium perenne* L.) and red clover (*Trifolium pratense* L.) were independently rotated with potatoes so that it would be possible to determine their effects on the golden nematode population. All treatments were assigned to plots at random.

Irish Cobbler, an early variety, and Green Mountain, a late variety, were each grown yearly in 8 plots. To provide a direct comparison of the effect of the most effective chemical treatment with that of the 3-yr. rotations, soil in 4 of the Cobbler plots and 4 of the Green Mountain plots was treated with dichloropropene-dichloropropane (D-D) mixture at the rate of 450 lbs. per acre in the fall of 1947. The chemical was applied to a depth of 4 in. on 10 in. centers by means of a hand injector.

All crops were grown according to approved farm practices for Long Island. A winter cover crop of rye was grown in each of the corn, green bean, and potato plots.

In 1948 potatoes were grown in all 48 plots. The viable golden nematode population was estimated from counts of the immature females on roots of all 28 plants in each plot. The method used in making these determinations is described by Chitwood and Feldmesser (1).

The procedure of the second rotation experiment differed from the first only in that 12 rotations were included, more than one crop was included in a single rotation each year, and only the Green Mountain variety was included. This experiment included a number of vegetable crops commonly grown on Long Island in addition to several hay, pasture, and cover crops (Table 2). The combinations of vegetables grown on the same plot in one year are those commonly grown by Long Island growers on the same land during one growing season.

RESULTS.—In the first experiment each 3-yr. rotation resulted in a significant reduction in number of immature females on potato roots of the 1948 crop for both the Cobbler and Green Mountain varieties (Table 1). There was no significant difference among the different rotations. With one exception, roots from soil continuously cropped with potatoes and treated with 450 lb. of D-D mixture during fall, 1947, were infected with significantly fewer immature females than roots of plants from the rotations. The difference between the counts for the corn and Cobbler potato plots and those for the D-D treated plots approached the significant level. The 3-yr. rotations including the Green Mountain potato variety resulted in 68 per cent fewer viable nematodes than did continuous cropping

[1] Accepted for publication March 14, 1952.

TABLE 1.—*Crop rotation and soil fumigation in relation to the viable golden nematode population of the soil.*

Crop grown in 1946 and 1947	Av. no. immature females per gram of potato root in 1948	
	Green Mountain	Cobbler
Potatoes	319	298
Red Clover	128	73
Perennial Rye Grass	113	100
Green Beans	85	83
Corn	81	61
Potatoes[a]	12	12
L. S. D. .01 level	56	53

[a] DD treatment in September 1947.

with potatoes, and rotations with the Cobbler variety 73 per cent fewer. The D-D treatment caused a 96 per cent reduction.

In the second experiment the most striking result was that the number of immature females on roots of potatoes growing in plots with continuous potato production was significantly greater than the number on roots of potatoes in any of the plots in which rotation was practiced (Table 2). In general, fewer immature females were present on potato roots growing in plots in which grasses had been grown than on potato roots growing in plots in which other types of crop plants were grown. Plots planted with Kentucky blue grass, a crop not particularly suitable for Long Island, had the lowest average number of females on the roots of the 1948 potato crop.

DISCUSSION.—It is not possible to compare accurately the numbers of immature females on roots of different varieties which begin growth at different soil temperatures and grow at different rates, because the females are not at the same stage of development at any one harvest date. If different harvest dates had been used for each variety the comparison would have been invalid because of the difference in environmental factors, especially soil moisture.

When potatoes are grown in soil with high populations of this organism, insufficient potato roots are available to feed all the larvae (1). Therefore, in heavily infested soil fairly large variation in actual numbers of living nematodes in the soil would not cause corresponding changes in numbers of immature females on the roots. Consequently, differences between counts on the roots from the plots with continuous potato production and those in which rotation or chemical treatment was practiced probably are less than would have been obtained had actual counts of the viable encysted golden nematodes in the soil been made by microscopic examination. After infested land has been free from potatoes for several years, a high percentage of larvae appear unhealthy, appearing neither dead nor healthy. Considerable error is introduced in judging by microscopic examination whether these larvae are capable of attacking and developing in potato roots. For this reason it is thought that counts of immature females on roots provide the most satisfactory measure of population change for use in these experiments.

In the first experiment, the numbers of immature females on potato roots in plots in which perennial rye grass or corn were grown in rotation with potatoes were not significantly different from the number of females on potato roots in plots in which clover or green beans were grown. In the second experiment, rotating potatoes with each of several grasses resulted in fewer immature females on the potato roots than on potato roots grown in rotation with other types of crops.

Triffitt (7) noted a reduction in the viable larvae content of golden nematode cysts after certain grasses had been grown in infested soil. None of the grasses were attacked by the nematode. Triffitt further found that the diffusate from the roots of some of these grasses stimulated migration of larvae from golden nematode cysts up to $\frac{2}{3}$ the rate of migration into potato root diffusate. Kentucky blue grass and rough-stalked meadow grass were most effective. Perennial rye grass was slightly less effective in stimulating migration. Franklin (3) obtained conflicting results in tests of the migration of golden nematode larvae into diffusate from roots of corn. In 1 experiment out of 3 there appeared to be stimulation by the diffusate. Lownsbery (1950) in a laboratory experiment found no significant difference between the stimulating efficacy of corn, perennial rye grass, red clover, or green beans.

It is evident that more field and laboratory experiments are needed to determine the relative effect of grasses and other types of crop plants on the golden nematode population of the soil when grown in rotation with potatoes. The most important factor in planning a crop rotation on golden nematode-infested

TABLE 2.—*Crop rotation in relation to the golden nematode population of the soil*

Crops grown in 1946 and 1947[a]	Av. no. immature females per gram of potato root—1948 crop.
Potatoes (*Solanum tubersum* L.)	241
Green beans and parsnips (*Pastinaca sativa* L.)	124
Beets (*Beta vulgaris* L.)	100
Spinach (*Spinacia oleracea* L.), lettuce (*Lactuca sativa* L.), and radish (*Raphanus sativus* L.)	87
Cabbage (*Brassica oleracea* L.)	86
Rough stalked meadow grass (*Poa trivialis* L.)	83
Birdsfoot trefoil (*Lotus corniculatus* L.)	78
Sugar corn (*Zea Mays* var. saccharata Bailey)	69
Sugar corn and turnips (*Brassica Rapa* L.)	69
Sugar corn (sown thickly)	64
Timothy (*Phleum pratense* L.)	62
Orchard grass (*Dactylis glomerata* L.)	61
Kentucky Blue Grass (*Poa pratensis* L.)	45
L. S. D. .01 level	51

[a] Green Mountain potatoes grown in each plot in 1948.

land, however, is to grow the host crops, potatoes and tomatoes, as infrequently as possible.

SUMMARY

The effect on golden nematode populations of 4 3-yr. rotations of corn, green beans, red clover, and perennial rye grass with potatoes was compared with that of continuous cropping to potatoes under Long Island conditions. Viable nematode population following each rotation was significantly smaller than that following continuous potato production but there was no significant difference among the different rotations. In plots continuously cropped with potatoes, the number of immature females on 1948 potato roots in soil injected with 450 lb. of D-D per acre in the fall of 1947 was only 4 per cent that in untreated plots similarly cropped.

In a second rotation experiment 12 3-yr. rotations, including a number of vegetable, hay, and pasture crops, were compared with continuous potato production. The number of immature females on roots of the potato crop grown in each of the rotations was significantly less than that on roots of potatoes grown in plots in which continuous potato production was practiced. The reduction varied from 49 to 82 per cent with an average reduction of 68 per cent. There were significant differences among the numbers of immature females on roots of potatoes grown in rotation with the different crops. The crops resulting in the lower readings were grasses, which are not suitable as commercial crops on Long Island.

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THE MORA VIRUS DISEASE OF SWEET CHERRY[1]

J. A. Milbrath

In 1949 an abnormally late fruit-ripening condition of Napoleon (Royal Ann) sweet cherry was noted in an orchard near Salem, Oregon. The fruit on 4 trees ripened about 6 wk. later than the fruit on adjoining trees of the same variety. A branch of a pollinator variety had been grafted into each of these trees several years previously and may have been the source of the infection. However, several normal-appearing trees had been grafted in a similar manner. When buds from diseased trees were placed in normal Napoleon trees the disease was transmitted, and the conclusion was that the causal agent was a virus. The failure of the fruit to size and mature at the proper season suggests symptoms of western X little cherry. However, fruits from trees affected by western X little cherry remain small and never ripen normally. While fruits affected by this disease are small and immature at the usual harvest time, they eventually develop to normal size and ripen in a normal manner. Western X little cherry does not produce the leaf symptoms characteristic of this disease. The combination of symptoms are not characteristic of any other virus disease described on stone fruits; therefore, the common name "mora virus disease" is suggested because of the abnormal delay of a month or more in the ripening of the fruit.

SYMPTOMS ON ORIGINAL ORCHARD TREES.—*Foliage*—Leaves on limbs with late ripening fruit were yellowish-green in contrast to the deep green of leaves of adjacent normal trees. Severely affected branches showed no terminal growth, the leaves were reduced in size, cupped upward and inward (Fig. 1B), and were discolored by a blotch type of chlorotic mottle indefinite in outline. The foliage on the fruit spurs appeared rosetted and twisted.

Fruit.—The infected trees were first seen August 19, 1949, about 6 wk. after the normal cherry harvest for the district. None of the adjacent normal trees had any fruit remaining on the branches, but the infected trees were still loaded with fruit. The grower reported that the fruits on these trees were too immature and small to pick during the usual harvest period, but later most of the fruit developed until it was nearly normal in color and size. Some of the fruits were pointed and misshapen as illustrated in Fig. 1A.

These trees were observed again in 1951 and 1952 and showed the same effect on fruit and foliage as observed in 1949.

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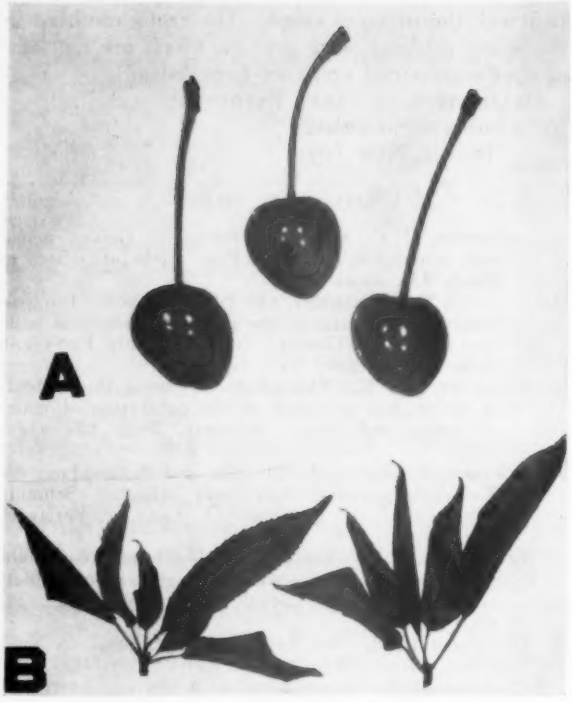

FIG. 1. Orchard symptoms of the "mora virus disease" on Napoleon sweet cherry. A. Late maturing, abnormally shaped fruits; B. Retarded growth and rolled leaves.

INOCULATION STUDIES.—Three Napoleon and 2 Bing trees were inoculated August 20, 1949 with 10 buds each from the infected orchard trees. None bore fruit in 1950 because of spring frost damage to the orchard. One of the inoculated Napoleon trees showed retardation of growth and rolling of leaves similar to that of the original trees. The Bing trees which were apparently virus free before inoculation, showed severe lace leaf symptoms on the inoculated branches, apparently due to a ring spot virus contamination. These inoculated branches showed only a mild lace leaf reaction or none at all in 1951, but the leaves rolled similarly to the diseased Napoleon leaves. There was very little fruit on the Bing trees, but the Napoleon tree which had shown infection the previous year set a good crop which was noticeably later in ripening than the non-inoculated check trees.

On September 21, 1950 5 more Napoleon trees and 4 more Bing trees, all known to be infected with ring spot virus, were inoculated with 10 buds each from the diseased Napoleon which was inoculated in 1949. By May 10, 1951 the inoculated branches on 3 of the Napoleon trees and 1 Bing tree showed evidence of disease transmisison. The lateral growth just beyond the point of bud insertion was much reduced in growth and the leaves were twisted and cupped upward (Fig. 2A and B). This effect became progressively less severe away from the inoculation point and towards the terminal. The Bing tree was affected in the same manner as the Napoleon and there was no evidence of lace leaf on any of the trees inoculated.

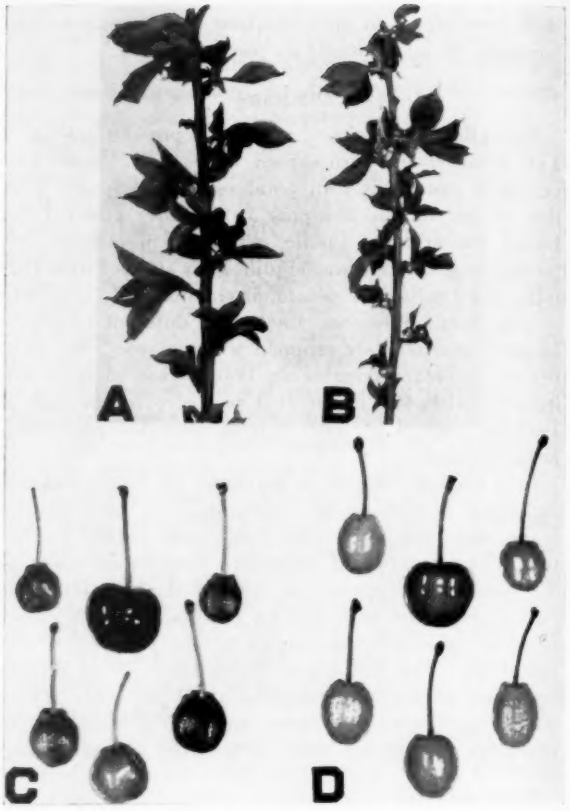

FIG. 2. First year or "shock" symptoms on trees inoculated with the "mora virus disease"; A. Inoculated branch from Bing and B. from Napoleon as they appeared in May following fall inoculation. C. Fruit from inoculated Bing branch and D. from Napoleon branch first year following inoculation. A normal fruit in center of each group. Note the small size, late maturity, roughened surface and collar around pedicel. (Photographs by H. H. Millsap.)

Many of the leaves on the inoculated branches and on some of the adjacent branches later developed rolled and cupped leaves similar to those observed on the original trees.

The fruit symptoms on these inoculated branches were even more striking. The ripening was retarded more than a month, and the developing fruits were elongated with a raised collar around the pedicels. The surface of the cherry was roughened and resembled the surface of a lemon (Fig. 2C and D).

The severe effect noted on both the foliage and fruit, which was not noticed on the original trees suggests a shock reaction as the host is first invaded. Later they produce the characteristic symptoms noted on the original orchard trees.

This virus disease appears to be potentially serious on sweet cherry but still is of limited distribution. No surveys have been made for the disease, but its nature is such that if it were generally distributed it would have been noted elsewhere.

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A VIRUS INHIBITOR IN CUCUMBER IN RELATION TO MOSAIC RESISTANCE [1]

W. H. Sill, Jr. and J. C. Walker

In 1929, Porter (6) showed that the oriental cucumber variety, Chinese Long, was highly resistant to cucumber mosaic incited by cucumber virus 1. This variety and another from the orient, Tokyo Long Green, have been used since as resistant parents in breeding projects in several states. In these projects commercially useful resistant varieties, such as Ohio 31 (10), Yorkstate Pickling (5), Niagara (5), Ohio MR17, and Kanmore, have been developed. While these varieties show marked freedom from symptoms during a major portion of the growing season, they each permit the establishment and increase of the virus within the plant. Tokyo Long Green and Chinese Long, under Wisconsin conditions, are symptomless except for infrequent, diffuse mottle in the expanded young leaves. The other resistant varieties mentioned show slightly more mottle in the terminal leaves and occasional mottle of fruits. By contrast, susceptible varieties show severe mottle of young leaves, stunting of internodes, and various degrees of mottle and distortion of the immature fruits. Shifriss, Myers, and Chupp (7) classified varieties on the basis of symptoms as susceptible, tolerant, resistant, highly resistant, and symptomless. Since a method has been suggested (9) by which the relative concentration of virus in different varieties and in different plant parts within a variety might be measured, it was desired to determine if there was a correlation between virus concentration and resistance. Early in the study a substance in cucumber tissue extracts highly inhibitive to the virus was discovered, necessarily altering the original line of approach. The present paper is concerned with the distribution of inhibitive substances in cucumber and in certain other species of plants and their possible relation to mosaic resistance.

METHODS AND MATERIALS.—The strain of cucumber virus 1 used for all assays was that designated in University of Wisconsin cultures as Y. It was isolated originally from spinach in southeastern Wisconsin and was used in an investigation of an inhibitor in spinach by Kuntz and Walker (3). Virus assays were made by use of the local-lesion response of primary leaves of Black cowpea, according to methods described elsewhere (9). Briefly, this consisted of using a 1 to 10 dilution of the extract from the virus-source plant in pH 7 phosphate buffer applied with the aid of carborundum to primary leaves of young cowpea plants which had been kept in the dark for 24 hr. immediately preceding inoculation.

Seeds of susceptible varieties of cucumber were obtained from commercial sources. Those of resistant Kanmore and Jeep varieties were from O. H. Elmer, Kansas State College; of resistant Ohio 31 from J. D. Wilson, Ohio Agricultural Experiment Station; of resistant MR20 and Ohio MR17 from H. J. Heinz Co.; of Yorkstate Pickling and Tokyo Long Green from H. M. Munger, Cornell University; and of Chinese Long from S. P. Doolittle, U. S. Department of Agriculture.

EXPERIMENTAL RESULTS.—Thirteen varieties of cucumber were inoculated in several tests to determine their relative resistance to strain Y as evidenced by development of symptoms. These were Chinese Long, Tokyo Long Green, Kanmore, Jeep, MR20, Ohio MR17, Ohio 31, Yorkstate Pickling, Producer, Maine No. 2, Stay Green, Chicago Pickling, and National Pickling. The first 2 were nearly symptomless. The next 5 were highly resistant but showed mottle of young leaves and infrequently of fruits. Yorkstate Pickling usually had a higher percentage of mottled leaves and fruits than the other 4. The last 5 varieties were very susceptible and showed severe stunting, extreme mottle of leaves, and frequent mottle and/or distortion of the immature fruits. Attempts were made, repeatedly but without success, to measure the relative concentration of virus in infected plants of each variety. When extracts of entire plants up to 18 in. in height were assayed few or no lesions appeared on cowpea plants. This held for highly resistant and for very susceptible, severely-diseased plants. In contrast inoculum prepared in the same manner from infected tobacco plants gave large numbers of lesions per leaf. The results from 8 representative trials in which the extract from infected cucumber plants was compared with an extract from infected tobacco plants gave an average of 3 lesions for the former and 93 for the latter. They indicated either a low concentration of virus in infected cucumber as compared with infected tobacco or the presence of an inhibitor in the extract of cucumber.

The usual 1 to 10 dilution of extract of infected tobacco in 0.1 M. phosphate buffer was compared with the same original extract diluted 1 to 10 with buffered juice extracted from healthy cucumber. In 11 trials the lesion average for the tobacco extract was 53 whereas that from the tobacco plus cucumber was 2. These results showed consistently that the cucumber extract contained a substance, or substances, which was highly inhibitive to the virus. This inhibitor may have been involved in the report of McKinney (4), who in 1927 stated that he observed a suppression in the percentage of tobacco plants infected systemically when cucumber rather than tobacco was used as the inoculum-source plant.

Extracts from healthy plants of 3 susceptible and 3 resistant varieties were assayed. With the exception of National Pickling 3 or more trials were made

[1] Accepted for publication March 20, 1952.
This report is from a joint project in the Departments of Plant Pathology and Biochemistry, University of Wisconsin, which is supported in part by a grant from the Research Committee of the Graduate School. Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

TABLE 1.—*Comparison of inhibitor potency in extracts from resistant and susceptible cucumber varieties*

Variety	No. of cowpea plants inoc.	Ave. lesions per leaf with extract from: tobacco diluted with buffer	Ave. lesions per leaf with extract from: tobacco diluted with buffered cucumber extract
National Pickling (S)[a]	23	57	12
Chicago Pickling (S)	28	44	1
Maine No. 2 (S)	34	67	5
MR20 (R)	39	91	4
Ohio 31 (R)	28	84	5
Kanmore (R)	36	50	2

[a] S = susceptible variety; R = resistant variety.

with each variety. Since the results were very similar for each variety the data for each are combined in Table 1. It was evident that the inhibitor was present in all varieties and that there was no correlation between mosaic resistance and degree of inhibition.

The relative potency of the inhibitor in various organs of healthy cucumber plants was determined by assaying extracts from green leaves, dead leaves, cotyledons, stem, entire etiolated seedlings, roots, fruits, seeds, entire blossoms, and corolla. The results presented in Table 2 showed that the inhibitive substance was present and highly potent in all parts except the corolla. There was an indication that the inhibitor was slightly less potent in stem, root, and fruit than in leaves, cotyledons, seeds, and entire blossoms. In a preliminary report by Sill (8) wherein it was stated that the corolla contained the inhibitor, the entire blossom was actually used. The present results show that the inhibitor is present in other parts of the blossom but that it is very low in or absent from the corolla.

Using the same technique extracts from healthy plants of watermelon, muskmelon, squash, pumpkin, cowpea, spinach, tomato, tobacco, and *Nicotiana rustica* were assayed for the presence of inhibitive substances. Watermelon and spinach extracts were as inhibitive as cucumber. The inhibitive effect of healthy spinach juice was reported previously by Grant (1) and by Kuntz and Walker (3). The extracts of other plants tested were inhibitive, but much less so than those from cucumber, watermelon, and spinach. Inhibition of virus infectivity varied from 19 per cent with *N. rustica* to 74 per cent with tomato. Indications of a mild inhibitive property in tobacco juice have been reported by McKinney (4) and in tomato extract by Johnson (2).

TABLE 2.—*Distribution of the inhibitor in various parts of the cucumber plant*

Plant Part	No. of cowpea plants inoc.	Ave. lesions per leaf with extract from: tobacco extract diluted with buffer	Ave. lesions per leaf with extract from: tobacco extract diluted with buffered plant part extract
Green leaf	13	16	1
Dead leaf	14	27	3
Cotyledon	12	141	7
Stem	25	69	13
Etiolated seedling	12	29	1
Root	23	110	18
Fruit	23	82	21
Seed	36	93	1
Entire blossom	14	31	6
Corolla	43	74	65

In most leaves of infected susceptible cucumber plants and in occasional young leaves of infected resistant plants symptoms consist of contrasting yellow and green areas. The percentage of yellow area is much higher in susceptible than in resistant varieties. The tissue of yellow areas was assayed in comparison with that of green areas of the same leaves. The number of lesions induced by extracts from yellow areas was very high and that from green areas very low. In 6 trials lesions from the first averaged 181 while those from the second averaged 1.5. These results could be interpreted as indicating that either the virus concentration was very high in yellow areas and very low in green areas, or that the inhibitor had not developed or had been reduced in amount in the yellow areas while normal in amount in the green areas. When surface tissues of yellow and green areas on infected fruits were assayed the same relation between lesion number and yellow or green tissue held as in the case of leaf tissues. There the lesion average from yellow areas was 222, from the green areas 13. When a similar comparative assay was made with normally and abnormally colored areas in fruits of pumpkin and squash, which species were shown earlier to be much lower in inhibitor content than cucumber, the differences were relatively slight.

When symptoms appear on infected plants the very young leaves are usually highly chlorotic with little or no green tissue. As the leaves expand, a mottle pattern develops in which yellow and green areas are distinguishable. This is true for susceptible and for resistant varieties with the exception of Chinese Long and Tokyo Long Green. In the last 2, chlorotic tissue is sparse and diffuse and it does not appear ordinarily until the leaf has reached nearly maximum size. The next experiments were designed to determine whether the inhibitor was present in young chlorotic leaves and whether in mottled leaves there was sufficient inhibitor present in green areas to reduce effectively the local-lesion count when the whole leaf was assayed. Several resistant and susceptible varieties were used. Whole young chlorotic leaves, except in the case of Tokyo Long Green and Chinese Long, whole mottled leaves, and yellow and green areas from the latter were assayed. The results presented in Table 3 showed that there was little or no inhibitor in the young chlorotic leaves, while it was very high

TABLE 3.—*Inhibitor potency of extracts of very young chlorotic leaves, older mottled leaves, and from yellow and from green areas in the latter*

Variety	Ave. local lesions per leaf with extract from whole young chlorotic leaves	whole older mottled leaves	yellow areas	green areas
Chi. Pickling (S)[a]	142	3	197	1
Nat. Pickling (S)	144	9	163	1
Stay Green (S)	165	12	265	1
Yorkstate Pickling (R)	106	1	216	1
MR 20 (R)	128	7	99	1
Ohio 31 (R)	57	1	177	1
Tokyo Long Green (R)		0	28	5
Chinese Long (R)		3	12	1

[a] S = susceptible variety; R = resistant variety.

in the extract of whole mottled leaves. When the yellow and green areas of the mottled leaves were assayed separately the inhibitive action was high in the green area extract but not in the yellow area extract with the exception of Tokyo Long Green and Chinese Long. The low lesion count with extracts from the yellow areas of the last 2 varieties was attributed to the fact that the yellow tissue is sparse and poorly defined in these varieties and satisfactory separation of the 2 areas is not possible. The high inhibitive action of the extracts from whole mottled leaves was regarded as indicative of the fact that there was sufficient inhibitor in the green portions to produce a high inhibitive action in the extract of the entire leaf.

When the corollas of infected cucumbers were assayed the highest lesion counts obtained at any time were secured. In Table 4 are the results of comparisons of assays of extracts of entire plants, yellow areas from mottled leaves, and corollas. Several resistant and susceptible varieties were used. As already shown in previous experiments the inhibitive action of extracts from entire plants was high while that from yellow leaf areas was very low with the exception of Tokyo Long Green and Chinese Long in which separation of yellow areas was difficult. In all varieties, however, the local-lesion count with extracts of corollas was very much higher than that from extracts of yellow areas in mottled leaves. This indicated that although the inhibitor was low in yellow leaf areas it was probably present in some degree while in corollas it was absent or infinitely lower than in yellow leaf tissue. It is particularly important to note that although it was impossible to secure a highly infectious extract from yellow areas in Chinese Long and Tokyo Long Green the corollas of infected plants of those varieties showed high virus concentration.

TABLE 4.—*Comparison of inhibitor potency in extracts of whole plants, yellow leaf tissue, and corollas of resistant and susceptible varieties*

Variety	Ave. local lesions per leaf with extracts from whole plant	yellow leaf areas	corolla
Chi. Pickling (S)[a]	3	290	403
Nat. Pickling (S)	5	137	342
Kanmore (R)	4	145	321
MR20 (R)	2	99	312
Tokyo Long Green (R)	1	28	363
Chinese Long (R)	1	5	204

[a] S = susceptible variety; R = resistant variety.

DISCUSSION.—This investigation has shown that extracts of healthy cucumber plants contain a substance highly inhibitive to cucumber virus 1 as indicated by local-lesion assay. The inhibitor is widely distributed throughout the plant parts with the exception of the corolla. The inhibitor is also widely prevalent in infected cucumber plants. Since no method has as yet been devised to separate inhibitor and virus an accurate comparison of virus concentrations in whole infected plants of resistant and susceptible varieties has not been possible. Inasmuch as the virus occurs in high concentration in the corollas of the most resistant varieties there is no question that it moves or increases systemically throughout the plant. In chlorotic tissue of infected leaves the inhibitor is low, and high lesion counts on cowpea result from extracts of such tissue. When such tissue is compared with corolla tissue the lesion counts from the latter are still higher, indicating that some inhibitor still remains in yellow leaf tissue.

While there is as yet no satisfactory means of relating the occurrence of inhibitor to mosaic resistance in the host some indirect evidence may be inferred. Aside from the corolla, the only tissue from which virus can be extracted in high concentration is the chlorotic areas of infected leaves and fruits. It may be assumed either that the inhibitor develops less abundantly in this tissue or that once formed it is largely destroyed as the effect of the virus on host metabolism is expressed. There is a rough inverse correlation between the amount of chlorotic tissue characteristic of infected plants of a variety and the resistance of the variety to detrimental effects of the virus. It may be postulated then, as a working hypothesis, that the inhibitor has an effect upon the increase of the virus or upon the influence of the latter upon metabolism of the cucumber plant. In healthy plants the inhibitor is apparently as abundant in resistant as in susceptible plants, but in infected plants there is less inhibitor and more measurable virus in susceptible than in resistant plants. Conversely as the degree of resistance goes up from variety to variety it is the more difficult to recover the virus because of its closer association with the inhibitor. It will require separation and further study of the in-

hibitor to determine whether or not it is a factor in restraining the influence of the virus on the metabolism of the invaded host cell.

SUMMARY

In an attempt to study the relative concentration of cucumber virus 1 in resistant and susceptible cucumber varieties by use of local-lesion assay on cowpea it was discovered that healthy and infected cucumber plant extracts were highly inhibitive to the virus. The inhibitor was present in green leaves, dead leaves, cotyledons, stems, etiolated seedlings, roots, seeds, fruits, and entire blossoms of all varieties tested. It was absent from or present in negligible quantities in corollas. Extracts from young chlorotic leaves and from yellow areas of mottled leaves showed high virus concentrations of the virus, owing to, presumably, a small amount of inhibitor. Extracts from green areas in mottled leaves and in mottled fruits, and from entire mottled leaves of infected cucumber plants were highly inhibitive. Extracts from healthy watermelon and spinach plants were highly inhibitive; those from muskmelon, squash, pumpkin, cowpea, tobacco, tomato, and *Nicotiana glutinosa* were moderately or slightly so. Since no means was discovered whereby virus and inhibitor could be separated the virus concentration could not be ascertained except in chlorotic areas and in the corolla. While it was not possible to determine the relation of the inhibitor to resistance it was significant that in diseased plants there was a rough inverse correlation between the amount of chlorotic tissue, in which inhibitor content was low and virus concentration high, and the degree of resistance of the variety.

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A PHYTOPATHOLOGICAL NOTE

A Method for Clearing Leaves.[1] CURT LEBEN.

In seeking a rapid method for removing pigments from tomato leaves so that the germination of spores of *Alternaria solani* (E. and M.) Jones and Grout on the leaves could be observed, it was found that effective decolorization was accomplished in less than an hour by extraction with pyridine. The spores and hyphae of the fungus were plainly visible microscopically and appeared not to have been changed by the treatment. The method has been used to clear the leaves of other plants and to observe the germination and penetration of other fungi, notably certain rust organisms. It seems to be of sufficient usefulness to warrant a brief description.

A small leaf section (e.g., one cm.^{2}) was placed in 10-20 ml. of pyridine and allowed to soak until the pigments were removed. This process could be hastened by using several successive pyridine soakings. The cleared section was mounted directly in lactophenol for microscopic examination, or it was washed first with water and then mounted. Various dyes (e.g., cotton blue) have been used, either before mounting or in lactophenol.

In the cleared leaf, the gross morphology of such parts as stomata, leaf hairs, vascular tissues, the upper and lower epidermis, and the mesophyll area appeared to have been preserved without marked change. In some specimens, pigment removal was so effective that it was possible to focus downward through the leaf to the lower epidermis where germinating spores and structural details of this area of the leaf could be observed.

The pigments have been removed by the pyridine method from the leaves of young bean, cabbage, corn, cucumber, oat, radish, strawberry, tobacco, and wheat plants. Extraction was not so effective with old leaves, especially from woody plants. Pyridine has also been used successfully to decolorize young tomato stems.—Department of Plant Pathology, University of Wisconsin, Madison, Wisconsin.

[1] Approved for publication by the director of the Wisconsin Agricultural Experiment Station. The author wishes to thank D. C. Arny and Gordon R. Grimm for their assistance. Tests with tomato stems were made by Robert P. Scheffer.

USING HALF-TREE PLOTS FOR INCREASING THE EFFICIENCY OF FUNGICIDE TESTS [1]

Saul Rich

Making comparisons on the 2 opposite halves of the same biological unit has been successfully employed in the half-leaf technique used in virus studies. The basic premise is that the 2 halves of the same biological unit are much less heterogeneous in their response than are 2 separate individuals. Not only are the 2 halves genetically identical, but environmental and nutritional differences are greatly reduced. The minimizing influence of extraneous variables in biological comparisons increases our ability to discriminate treatment differences. It was of interest, therefore, to design experiments comparing fungicide trials in such fashion as to include the advantages inherent in the half-leaf technique. Instead of testing them on half-leaves, however, fungicides for the control of apple scab were compared in the field by the use of half-tree plots. Different treatments were applied to opposite halves of the same tree. In the case of apple trees, this technique minimized climatic, nutritional, and historical differences only, as genetic differences were not involved. Besides making for better comparisons, the half-tree technique had the important added advantage of doubling the number of treatments which could be tested.

METHODS.—The treatments were applied to the east and west halves of 28 standard McIntosh trees about 15 to 20 ft. high. The trees, running in north-south rows were divided into 4 replicates. The 14 treatments were randomized within each replicate. The only requirement imposed on the randomization scheme was that in each treatment 2 of the plots faced east and the other 2 faced west. This arrangement made it convenient to analyze for any east-west effect.

The desired concentrations of the fungicides being applied were carried in 55 gal. drums placed on a flat-bedded truck, along with the hydraulic spray equipment. The spray pressure was 300 lbs. per sq. in. and the spray cone was controlled by the use of a Bean Spraymaster spray-gun. Proper and continual adjustment of the spray cone controlled the depth to which the spray stream penetrated, and made possible the selective spraying required. It was possible to change treatments by shifting the intake hose from one drum to another. Clean water was passed through the spraying system between treatments. With this technique, the number of drums which could be carried on the truck was the only limit on the number of treatments which could be applied during 1 trip through the orchard. The plots were sprayed on each of the following dates: April 24, May 3, May 14, May 25, June 7, and June 21.

The data (Table 1) were taken on July 10, and represent the average leaf area covered by scab. As this is not a discussion of the relative merits of fungicides, the treatments are indicated by letter only. Fifty leaves were read from each plot for a total of 200 leaves per treatment. The Horsfall-Barratt[2] system of estimation was used. The leaf samples were taken from the east-west "faces" of the trees. Any areas of possible spray overlap, such as the north-south borders of the plots, were carefully avoided when taking leaf samples.

TABLE 1.—*Disease data from half-tree plots*

Treatment	Pounds of formulation per 100 gal.	Mean index[a] of disease	% of leaf area[a] showing apple scab
A	1	5.42	48.4
A	4	1.11	2.5
B	½	1.32	3.1
B	2	0.76	1.2
C	2	2.68	9.8
C+X	2+1	2.67	9.7
D	½	2.90	11.5
D	2	1.30	3.0
D+X	½+¼	2.83	10.8
D+X	2+1	1.17	2.8
E	½	0.27	0.5
F	1+3	2.39	7.9
G	4	5.10	42.0
H	nil	5.95	58.7

[a] Based on Horsfall-Barratt grading system.

RESULTS AND DISCUSSION.—An analysis of variance segregated the block error and showed highly significant treatment differences. The resulting error term was compared to the error term for a set of data resulting from using the single tree method on the same orchard in a previous year. When allowance was made for differences of variance caused by sample size, the 2 error terms did not differ significantly. Information is, therefore, approximately the same for both methods.

An analysis of covariance[3] showed that it made no significant difference whether a plot faces east or west. Apparently any environmental differences induced by east-west location were not sufficiently marked to be detectable.

Two of the more important considerations involved in these experiments were possible cross-tree contamination by 1) spray drift and 2) systemic effects. If either of these were important, then the best treatments should consistently reduce the amount of disease in their opposite plots. This effect would appear as a regression when the amount of disease in good treatments (less than 9 per cent disease) was plotted

[1] Accepted for publication March 21, 1952.
The author is indebted to Dr. A. E. Dimond for suggesting this technique.

[2] Horsfall, James G. Fungicides and their Action. 239 pp. Chronica Botanica, Waltham, Mass. 1945.

[3] This analysis was made by Dr. Paul E. Waggoner.

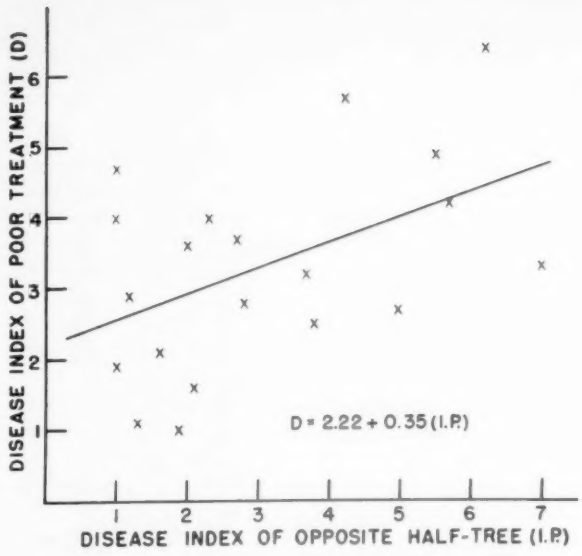

FIG. 1. Effect of inoculum potential (I. P.), expressed as disease index of opposite half-tree, on disease index of poor treatments (D). These data gave a significant "t" value of 2.18.

against the amount of disease in their opposite half-trees. The analysis showed that there was no regression, and hence spray drift contamination and systemic effects did not appear. Freedom from contamination by spray drift may be explained on the basis of the work of Potts[4] and others. They showed that very fine droplets could not get to the leaf unless the droplets had sufficient velocity to penetrate the air layer immediately adjacent to the leaf surface. As it is only the smaller droplets which would drift, it is probable that droplets in spray drift cannot settle on leaves in numbers sufficient to cause detectable contamination.

Graphic analysis of the data showed a possible relationship between the poor treatments (more than 9 per cent disease) and the amount of disease in their opposite half-trees. This indicated that the poor treatments were being affected by the amount of inoculum in the opposite half-trees. The greater the inoculum, the greater the amount of disease appearing in the poor treatments. Analyses of correlation and regression were made on these data excluding the untreated checks. The unsprayed checks, serving as early sources of secondary infection, acquired their disease load early, and could not have been appreciably affected by secondary infection caused by inoculum originating in opposite plots. The data gave a significant correlation coefficient of 0.46. If considered by means of the regression equation (Fig. 1), the effect of inoculum potential accounted for about 1/5 of the variation in disease on plots receiving poor treatments.

The inoculum potential effect is detectable in the half-tree technique because of the close proximity of the plots located on the opposite halves of the trees. This source of variation, using adjacent trees as a measure of inoculum potential, was not a detectable factor in the single tree method. It is important, therefore, that the influence of inoculum potential on poor treatments be recognized as a weak but significant source of variation inherent in the half-tree technique. The difference in importance of the inoculum potential between the 2 methods indicates that secondary infection resulting from the conidia produced within the leaf canopy is much greater than infections from conidia produced on adjacent trees. This is to be expected from spores such as apple scab conidia which move principally with splashing rain drops and dripping water rather than being largely wind-borne.

SUMMARY

The use of half-tree plots does not decrease the amount of information concerning treatment differences, and in addition increases the number of treatments which can be tested. The half tree method also gives a more reliable estimate of error because it is based on a greater number of degrees of freedom. This in turn allows for discriminating smaller differences between treatments. With a moderate amount of care in spraying, there is little chance of cross-tree contamination by spray drift. As long as spray drift is not important, this technique may be used to detect cross-tree influences caused by systemic effects.

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[4] Potts, S. F. Particle size of insecticides and its relation to application, distribution, and deposit. Jour. Econ. Entomology 39: 716-720. 1946.

SOME RELATIONSHIPS OF THREE PLANT VIRUSES TO THE DIFFERENTIAL GRASSHOPPER, *MELANOPLUS DIFFERENTIALIS* (THOS.)[1]

H. J. Walters [2]

It is generally believed that most of the known insect-transmitted viruses are carried by sucking insects and that chewing insects play a relatively small part in virus transmission. The fact that certain sap-transmissible viruses are spread so rapidly without the aid of a known vector indicates that something more than purely mechanical transmission might occur. The possible relations of these viruses to insect vectors have been comparatively neglected, since they can be easily propagated and their properties determined without use of vectors.

There are only a few authentic records of insects with chewing mouth parts acting as vectors. The viruses of cucumber mosaic, spindle tuber and unmottled curly dwarf of potato, turnip yellow mosaic, and cowpea mosaic have been transmitted by chewing insects (9, 13, 25, 30).

Insect transmission of tobacco mosaic virus has been investigated by several workers whose studies have shown conflicting results. Allard (1, 2) was the first to report transmission of a virus, thought to be tobacco mosaic, from tobacco to tobacco with aphids. Later Hoggan (16) suggested that Allard was working with cucumber mosaic virus. Hoggan (17, 18, 20) failed to transmit tobacco mosaic virus from tobacco to tobacco with aphids, but did transmit it from tomato to tobacco and other solanaceous hosts. Gigante (12) also reported aphid transmission of the virus. Elmer (10), Olitsky (27) and Sulkov and Vovk (36) reported transmission of tobacco mosaic virus with other insects. Attempts to transmit the virus with *Angytatus volucer* Kirk and *Thrips tobaci* Lindeman were unsuccessful (21, 29).

Smith (32) and Koch (23) were unable to transmit potato virus X with *Myzus persicae* (Sulz.). Smith (32) states that none of the common sap-sucking insect fauna of the potato plant is able to act as a vector, but suggests that the virus may be transmitted by a flower-feeding species of thrips. Cockerham (8) found no correlation between the flowering habit of potatoes and the presence of X virus.

Fenne (11) was unable to transmit tobacco ringspot virus with several insects frequenting tobacco. Hoggan (19) failed to transmit the virus to spinach with aphids. Pound (28) was unable to transmit a yellow strain of tobacco ringspot virus responsible for a mosaic disease of watermelons from tobacco to other hosts with *Myzus persicae*.

Earlier studies on the relationships of viruses to insect vectors showed that juices of macerated insects usually inhibit the infectivity of viruses (5, 14, 31). McClintock and Smith (26) reported that inoculations with juice of virus-bearing aphids produced infection with the virus causing spinach blight. Smith (34) reported that blood of *Protoparce sexta* (Johan.) inhibited the infective power of tobacco mosaic and tobacco necrosis viruses, and that certain viruses were destroyed within the digestive tract of the caterpillar.

Grasshopper transmission of plant viruses has been reported only twice previously (13, 25). Transmission of the viruses of tobacco mosaic, potato X and tobacco ringspot by grasshoppers discussed in this paper was published earlier in a preliminary report by the author (37). It was hoped that the present investigation might yield additional information concerning certain relations of insects with chewing mouth parts to the viruses they transmit.

MATERIALS AND METHODS.—The original stock of the differential grasshopper, *Melanoplus differentialis* (Thos.), was obtained from eggs taken from fields, and incubated until hatched at 80° F. in small containers partly filled with moist soil. To maintain the supply eggs were taken from soil, incubated approximately 7 wk. at 70° to 80° F. for embryo development, and stored at 40° F. The insects, kept in plastic screened cages at 90° F., were fed alfalfa and young corn while immature. As adults, the stage used for all tests, they were fed only corn.

For transmission experiments grasshoppers were starved from 4 to 6 hr. before an infection feeding, transferred by hand, 1 to each test plant, and released. Precautions were taken to prevent contamination in transferring insects and to avoid infection from other sources. For tobacco mosaic virus, the hybrid plant, *Nicotiana tabacum* L. x *N. glutinosa* L., was usually used (22), but a few tests were made with tobacco, *N. tabacum*. Tobacco was also used as test plant for potato virus X and tobacco ringspot virus. Unless otherwise stated, each grasshopper was moved to a new location on the test plant after taking a few bites or making a hole in the leaf about 1 to 6 mm. in diameter.

The dark brown fluid emitted from grasshopper mouths, referred to in this report as buccal fluid, is undoubtedly made up of regurgitated substances from the insect's crop as well as salivary secretions (6). The fluid was collected with a tapered blood pipette, inserted between the insect's mandibles, and sucked into the pipette through a piece of small rubber tubing attached to its basal end. A composite sample of

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Based on a thesis submitted by the author in partial fulfillment of the requirements for the Doctor of Philosophy degree. The study was made possible by funds provided by the Biological Department, Chemical Corps, Camp Detrick, Frederick, Maryland, under Contract No. W-18-064-CM-226 with the University of Nebraska, and was supervised by Dr. W. B. Allington, Department of Plant Pathology.

[2] Assistant Plant Pathologist, Agricultural Experiment Station, University of Wyoming, Laramie, Wyoming.

fluid from several insects was used for each experiment. Grasshoppers were starved 3 to 4 hr. prior to collecting the fluid to avoid an admixture of plant extracts. The pH of buccal fluid was found to be about 5.6.

Blood was extracted in a similar manner by inserting a fine glass capillary between the hind leg and the abdomen of a grasshopper. A composite sample of blood from several insects was used in most tests, but blood of several individual grasshoppers was tested separately. The pH of blood was near 6.8. Where virus was injected into the bloodstream of insects, the method described by Heal and Menusan (15) was employed.

Inoculum was prepared from leaf tissue of tobacco infected with the virus of tobacco mosaic, potato X (ringspot strain), or tobacco ringspot. The leaves were ground up and the juice expressed through cheese cloth. Dilutions were made with distilled water.

Experimental plants were grown in composted soil at greenhouse temperatures of 75° to 80° F. Quantitative tests with tobacco mosaic virus were made using the local lesion reaction on *N. glutinosa*. Tobacco was used for potato ringspot virus, and the primary leaves of cowpea (*Vigna sinensis* Endl. var. Blackeye) usually were used with tobacco ringspot virus.

A Latin square design of half-leaf units was used in most infectivity comparisons of tobacco mosaic virus; in most tests with the other 2 viruses half-leaf comparisons were made on 1 or 2 leaves on 4 suitable plants. Inoculations were made by dipping the forefinger in the inoculum, rubbing the leaves dusted with 600 mesh carborundum, and then rinsing with tapwater. Except as noted, inoculations were completed within 2 or 3 min. after mixing virus and buccal fluid or blood of grasshoppers; each experiment was completed in 20-30 min.

EXPERIMENTAL WORK.—*Transmission Experiments.*—a) *Tobacco mosaic virus.*—Preliminary tests showed that 29 per cent of grasshoppers tested immediately after an infection feeding transmitted tobacco mosaic virus and that successful transmission occurred after 12 hr. of starvation following an infection feeding.

To ascertain how long grasshoppers remain infective, they were tested immediately, and after starvation periods of 2, 4, 8, 12, 16, 20 and 24 hr. following an infection feeding of 20 to 30 min. Twelve insects were used for each test and each insect was allowed to feed at approximately 10 different locations on individual test plants. Transmission of virus occurred in all tests (Table 1). The percentage of transmission did not vary to a great extent in tests made immediately after an infection feeding through the 12-hr. tests. Only 1 plant became infected in the 24-hr. test.

To determine if grasshoppers could transmit virus over a series of feedings on healthy plants, they were transferred to hybrid plants immediately after an infection feeding of approximately 2 min. Each insect was allowed to feed in 1 location on each half of 3 leaves on 3 plants, a total of 18 feeding areas on 9 leaves. Forty of the 48 grasshoppers tested transmitted the virus. The greatest number of lesions occurred adjacent to the first 6 feeding areas. In some cases lesions were consecutive, and in others there was lack of infection in as many as 16 feeding areas with infection occurring with subsequent feedings (Table 2).

When undisturbed, grasshoppers may feed for a short time in a location or may completely consume an entire leaf or plant. To make sure that under natural conditions these insects did not always consume tissue already inoculated, they were transferred to hybrid plants immediately after a 5-min. infection feeding and each insect was allowed to feed at will on individual plants from 4:00 P. M. to 9:00 A. M. the following day, a period of 17 hr. Feeding ranged from single penetrations of the leaf tissue with the mandibles to almost complete consumption of nearly all leaves on a plant (Fig. 1). Ten of 20 grasshoppers tested transmitted the virus. In a similar test with tobacco as the test plant, 9 of 20 insects transmitted tobacco mosaic virus. These results demonstrate transmission of tobacco mosaic virus by *Melanoplus differentialis* under conditions such as might occur in the field.

Local infection caused by tobacco mosaic virus on

TABLE 1.—*Transmission obtained with 12 grasshoppers tested at various intervals after an infection feeding on tobacco plants infected with tobacco mosaic virus*[a]

	Hours after feeding on the virus source							
	0	2	4	8	12	16	20	24
No. insects transmitting	10	8	9	8	7	6	2	1
Percentage of insects transmitting	83.3	66.6	75.0	66.6	58.3	50.0	16.6	8.3
No. lesions produced from 120 feeding areas[b]	5/17	5/16	6/19	4/13	5/9	4/7	1/2	0/1

[a] Grasshoppers were allowed an infection feeding time of 20 to 30 min. and 10 feeding areas on hybrid test plants.
[b] The numerator indicates the number of lesions that were not adjacent to known feeding areas, while the denominator shows the total number of lesions produced.

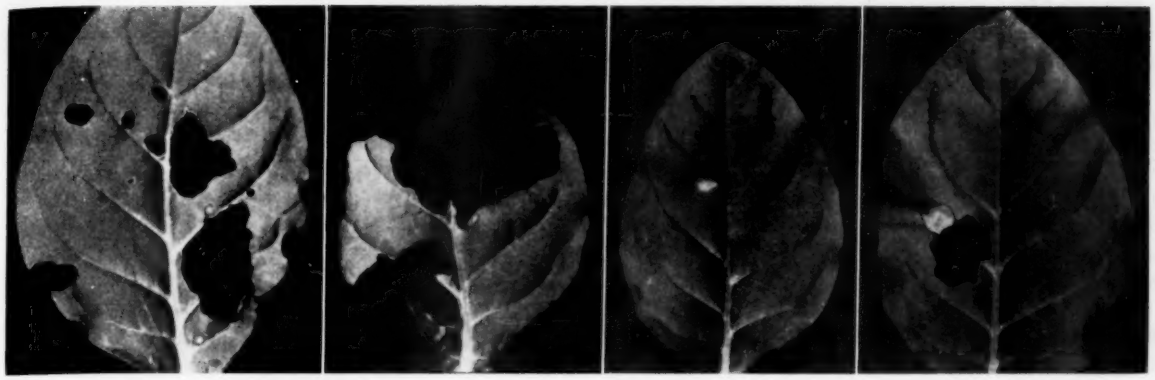

FIG. 1. Leaves of hybrid tobacco showing local lesions caused by tobacco mosaic virus after feeding of grasshoppers which ranged from a single penetration of leaf tissue to almost complete consumption of a leaf.

hybrid plants was not always adjacent to apparent feeding areas. Approximately 3 per cent of the total lesions occurred elsewhere. In most cases, a single lesion occurred adjacent to a feeding area, but in others as many as 3 lesions were evident. Feeding on a vein, or development of a lesion adjacent to a vein, brought about systemic infection of hybrid plants in several instances.

b) *Potato X and tobacco ringspot viruses.*—Grasshoppers were transferred to healthy tobacco plants immediately after an infection feeding of approximately 2 min. on tobacco infected with either the virus of potato X (ringspot strain) or tobacco ringspot virus. The insects were allowed to feed at 6 to 8 different locations on the test plants. Primary symptoms were evident on the test plants within 5 to 7 days. Later the plants became systemically infected. Eighteen of 100 grasshoppers tested transmitted potato virus X and 6 of 100 insects transmitted tobacco ringspot virus. With potato virus X 4 of the initial infections occurred away from apparent feeding areas, while with tobacco ringspot virus only 1 initial infection occurred away from a known feeding area. In a majority of cases, with both viruses, initial infection developed adjacent to feeding areas near 1 of the larger veins.

Effect of Buccal Fluid on Viruses.—Smith (30) obtained some infection with cowpea mosaic virus by inoculations with regurgitated juice from *Cerotoma trifurcata* Forst. which had previously fed on diseased plants. Markham and Smith (25) stated that there appears to be a correlation between the ability of the larva of *Phaedon cochleariae* F. to transmit turnip yellow mosaic virus and its regurgitation of some of the contents of the foregut. Results of the present investigation demonstrated that regurgitated substances (buccal fluid) of grasshoppers do not completely inhibit infectivity of tobacco mosaic, potato ringspot, and tobacco ringspot viruses.

Preliminary tests using equal amounts of each of the 3 virus extracts and buccal fluid inoculated to leaves of suitable plants showed that none of the viruses were completely inhibited. Buccal fluid was most inhibitive for tobacco ringspot virus, least for tobacco mosaic virus, and intermediate for potato ringspot virus.

a) *Effect of concentration of buccal fluid on viruses.*—Extracts of tobacco mosaic virus were diluted 1:1, 1:10, 1:50, and 1:100 with buccal fluid and compared with similar virus-water dilutions. Inoculations were made on leaves of *Nicotiana glutinosa.* Results of 5 tests are in Table 3. Infectivity of virus was decreased with increased concentration of buccal fluid. Almost complete inhibition of virus occurred at the 1:100 dilution. Potato ringspot and tobacco ringspot viruses were completely inhibited at dilutions greater than 1:1 with buccal fluid.

b) *Effect of dilution of the inhibitor.*—To test the

TABLE 2.—*Number of local lesions of tobacco mosaic virus produced on hybrid plants as a result of the feeding of 40 individual grasshoppers in a series of 18 feedings*[a]

Location of lesions	Feeding Number																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Adjacent to feeding areas	11	9	7	11	10	9	4	0	5	0	3	0	5	1	1	1	5	2
Away from feeding areas	2	0	3	0	4	2	0	0	0	0	0	0	1	0	0	0	0	0
Total lesions	13	9	10	11	14	11	4	0	5	0	3	0	6	1	1	1	5	2

[a] 48 grasshoppers were tested of which 40 transmitted tobacco mosaic virus.

TABLE 3.—*Effect of concentration of buccal fluid on infectivity of tobacco mosaic virus*

Tests	*Average[a] number lesions per half-leaf on N. glutinosa produced by 1 part virus in* 1 part buccal fluid		10 parts buccal fluid		50 parts buccal fluid		100 parts buccal fluid	
	Treated	Control[b]	Treated	Control[b]	Treated	Control[b]	Treated	Control[b]
1	36.0	248.3	10.0	171.0	1.8	130.3	0.8	89.8
2	34.0	269.3	4.5	160.8	1.8	95.8	0.3	67.0
3	20.8	179.8	3.0	106.8	0.0	70.3	0.0	47.3
4	31.0	218.0	4.3	149.5	0.8	123.5	0.0	60.0
5	17.0	181.8	2.0	84.5	0.3	61.0	0.3	34.5

[a] Average of 4 half-leaves for each test.

[b] Controls consisted of similar dilutions of virus and water.

effect of dilution of the inhibitor in buccal fluid a basic mixture of the fluid and extracts of each of the 3 viruses was prepared. For tobacco mosaic virus the basic mixture consisted of 1:10 virus extracts in buccal fluid, while for potato ringspot and tobacco ringspot viruses a mixture consisting of equal parts of the desired virus extract and fluid was used. These mixtures were diluted 1:10, 1:100, and 1:1000 with water and the 4 mixtures were compared with similar virus-water dilutions. The tobacco mosaic virus-buccal fluid mixtures were inoculated in a Latin square design to *N. glutinosa* and the mixtures of potato ringspot and tobacco ringspot viruses to 8 half-leaves of tobacco and cowpea respectively. Results of these tests are in Table 4. With tobacco mosaic virus and tobacco ringspot virus a definite increase over the infectivity of the original mixture in number of lesions occurred with dilution up to 1:100, but infectivity was greatest at dilution of 1:10. With potato ringspot virus, all dilutions increased infectivity but the greatest infectivity occurred at dilution of 1:100.

c) *Effect of heat on the inhibitor.*—Temperature treatments were carried out on mixtures of virus extracts and buccal fluid and compared with similar virus-water mixtures. For tobacco mosaic virus, a mixture of 1 part infectious plant extracts in 10 parts buccal fluid was diluted 1:10 with water. A mixture of equal amounts of buccal fluid and virus extracts was diluted 1:10 with water for potato ringspot virus, while the mixture for tobacco ringspot virus was 1 part buccal fluid in 100 parts virus extracts diluted 1:10 with water. Treatments were made by immersing 75 x 10 mm. test tubes, containing 1 cc. of mixture, in a water bath held within 0.1° C. of the desired temperature for 10 min. The preparations for tobacco mosaic virus were treated at 60°, 65°, 70°, 75°, and 80° C. and compared with a preparation kept at room temperature (23° C.), while those for both potato ringspot and tobacco ringspot viruses were treated at 55°, 60°, and 65° C. At the end of the 10-min. treatment, the tubes were immersed in cold water. Inoculations were made to suitable host plants after each series of temperature treatments was completed. Infectivity of the mixture of tobacco mosaic virus extracts and buccal fluid increased with rising temperature with highest infectivity at 70° C. A greater number of lesions occurred at 75° C. than with the preparation kept at room temperature, but infectivity of the suspension treated at 80° C. was markedly lower.

Infectivity of the 55° C. treatment of both the

TABLE 4.—*Effect of dilution on mixtures[a] of buccal fluid and each of 3 viruses.*

Virus	*Average[b] number of lesions per half-leaf produced on suitable hosts[c] by virus-buccal fluid mixtures at the following dilutions* No dilution		1:10		1:100		1:1000	
	Treated	Control[d]	Treated	Control[d]	Treated	Control[d]	Treated	Control[d]
Tobacco mosaic	13.6	186.9	24.0	95.4	16.3	27.0	5.4	3.8
Potato ringspot	2.8	295.7	8.0	172.6	12.4	84.1	8.1	24.8
Tobacco ringspot	13.8	117.4	41.7	91.8	33.0	59.7	4.9	2.6

[a] Mixtures of buccal fluid and tobacco mosaic virus consisted of 1 part virus to 10 parts buccal fluid, while mixtures of potato ringspot virus and tobacco ringspot virus and buccal fluid consisted of equal amounts of virus extracts and buccal fluid.

[b] Average of 20 half-leaves for tobacco mosaic virus and 40 half-leaves for potato ringspot and tobacco ringspot viruses in 5 experiments.

[c] *Nicotiana glutinosa* was host for tobacco mosaic virus, tobacco for potato ringspot virus, and cowpea for tobacco ringspot virus.

[d] Control consisted of similar dilutions of virus and water.

buccal fluid-virus mixtures and control for potato ringspot and tobacco ringspot viruses was below that kept at room temperature with infectivity completely suppressed at 60° C. There was no indication of the effect of heat on the inhibitor in preparations of potato ringspot and tobacco ringspot viruses since infectivity of the 2 viruses was destroyed at 60° C.

d) *Effect of dialysis.*—Attempts were made to separate the buccal-fluid inhibitor from a mixture of tobacco mosaic virus extracts and buccal fluid by dialysis through a cellophane membrane. A mixture of 1 part virus extracts to 10 parts buccal fluid was diluted 1:50 with water and dialyzed against running tap water, along with a similar virus and water preparation, for 48 hours. A nondialyzed sample of both preparations was stored at 2° C. for the same period of time and the materials compared on half-leaves of *N. glutinosa*. Results of 3 trials showed no increase in number of lesions produced by the buccal fluid-virus mixture which was dialyzed over that not dialyzed. The inhibitor in buccal fluid is not readily dialyzable through a cellophane membrane. There was a slight increase in number of lesions produced with the dialyzed virus-water preparation over that not dialyzed. This was probably due to dialysis of some inhibitive agent present in plant extracts.

Recovery of Virus after Ingestion by Grasshoppers.—Markham and Smith (25) found that turnip yellow mosaic virus was retained in an infective condition for 4 days in larvae of *Phaedon cochleariae*. Viruses of tobacco mosaic, potato ringspot, and tobacco ringspot were found to remain infective in buccal fluid within grasshoppers for several hours. A sample of buccal fluid was extracted from each of 12 insects at different time intervals, following a 30-min. infection feeding on tobacco infected with the desired virus, and inoculated to suitable hosts for each virus. *Nicotiana glutinosa* was used for tobacco mosaic virus and tobacco for the other 2 viruses. All samples were infective immediately after the insects fed, but their infectivity decreased as the interval between feeding and extraction of buccal fluid increased. Tobacco mosaic virus remained infective in 3 insects for 12 hr., while potato ringspot virus remained infective in 5 insects for 6 hr. and tobacco ringspot virus in 3 insects for 4 hr.

Negative results were obtained in an effort to ascertain whether the virus of tobacco mosaic entered the blood of grasshoppers after they had fed on virus-infected plants. At 24-hr. intervals, up to 10 days, after feeding for an hour on infected tobacco, a sample of blood was extracted from each of 20 grasshoppers and inoculated to 1 leaf of *N. glutinosa*. All attempts to demonstrate the presence of virus in the blood were unsuccessful.

Tobacco mosaic virus has been shown to pass through the digestive tract of *Protoparce sexta* unchanged though greatly reduced in concentration (34). To ascertain whether this virus is inactivated within the digestive tract of grasshoppers, 12 insects were starved for 24 hr. and then allowed to feed on mosaic-infected tobacco over a period of 3 days. Fresh feces were collected at different intervals of time during the feeding period, suspended in water, and inoculated to 8 *Nicotiana glutinosa* leaves. An average of 189, 275, 222, 292, and 202 local lesions per leaf was produced by collections made at 5, 20, 24, 48, and 72 hr. respectively. Similar tests were conducted with grasshoppers fed on plants infected with either potato ringspot virus or tobacco ringspot virus and the fecal suspension inoculated to 6 leaves of healthy tobacco. An average of 17, 24, and 19 lesions were produced by fecal suspensions of potato ringspot virus collected 24, 48, and 72 hr. respectively. Lesions did not occur on all leaves inoculated with fecal suspensions of tobacco ringspot virus. Only 2 lesions occurred on 1 of 6 leaves inoculated with the 24-hr. collection. Negative results were obtained with the 48-hr. collection and only 1 lesion was produced on 1 of 6 leaves inoculated with the 72-hr. collection.

To determine concentration of tobacco mosaic virus present in naturally voided feces a suspension consisting of equal amounts by weight of feces and water was inoculated to 5 half-leaves of *N. glutinosa* and compared with a 1:10 dilution of extracts from plants on which the grasshoppers had fed. Suspensions of fecal collections made at 24, 48, and 72 hr. produced an average of 221, 239, and 137 lesions per half-leaf respectively, as compared with 157, 243, and 143 lesions produced by diluted plant extracts. Tobacco mosaic virus passing through grasshoppers was not greatly reduced in concentration, but was comparable to that of a 1:5 dilution of infectious plant extracts.

Injection of Tobacco Mosaic Virus into Blood of Grasshoppers.—Injection of tobacco mosaic virus into the blood of living insects has been reported to inhibit the infectivity of the virus (34). To study the effect of blood on tobacco mosaic virus, 0.02 cc. of infective plant extracts were injected into the blood stream of each of 12 grasshoppers between the third and fourth abdominal segments, 0.01 cc. on each side. The hypodermic needle was inserted just beneath the integument to prevent entry of virus into the alimentary tract. A blood sample was extracted from each insect at 24-hr. intervals for 7 days and on the tenth and fifteenth day after injection and inoculated to 1 *N. glutinosa* leaf. Results of these tests are in Table 5. At least 91 per cent of the blood samples taken up to 120 hr. after injection were infective and 50 per cent after 10 days, but after 15 days virus was detected in blood of only 1 sample.

To determine whether tobacco mosaic injected into the blood of living grasshoppers would enter the buccal cavity, a sample of buccal fluid collected from each of 12 insects was inoculated to 1 leaf of *N. glutinosa*. A total of 5 lesions was produced from 3 of 12 samples taken 4 hr. after injection and a total of 7 lesions from 3 samples was produced after 8 hr. Negative results were obtained with samples collected 12, 24, 48, 72, and 96 hr. after injection. Apparently

TABLE 5.—*Infectious nature of individual blood samples of 12 grasshoppers taken at different intervals after an injection of tobacco mosaic virus into the blood stream*[a]

Time blood sample was taken after injection (hours)	Number samples infective	Average [b] number lesions produced on 1 *N. glutinosa* leaf per infective sample
24	12	36.0
48	11	18.0
72	12	8.8
96	12	27.0
120	11	13.8
144	9	4.7
168	7	2.1
240	6	3.8
360	1	1.0

[a] 0.02 cc. of infectious plant extracts was injected into each of 12 insects, 0.01 cc. on either side.
[b] An average of 1 *N. glutinosa* leaf in 1 trial.

some virus passed from the blood into the buccal cavity up to 8 hr. after the virus injection.

Effect of Extracted Blood on Viruses.—Smith (34) found that blood from *Protoparce sexta* when mixed with "fairly concentrated samples" of tobacco necrosis virus completely inhibited infection on *Phaseolus vulgaris* L. Preliminary results with extracted grasshopper blood showed that mixtures consisting of equal amounts of blood and virus extracts of tobacco mosaic, potato ringspot, or tobacco ringspot were not completely inhibited when inoculated to suitable hosts. Blood had the greatest inhibitive effect on tobacco ringspot virus, and had the least effect on tobacco mosiac virus. Infectivity of tobacco ringspot virus was almost completely inhibited on cowpea.

To study the effect of dilution, a basic mixture consisting of equal amounts of blood and extracts of tobacco infected with tobacco mosaic, potato ringspot, or tobacco ringspot virus was prepared and diluted 1:10, 1:100, and 1:1000. The basic mixtures and dilutions were compared with similar virus-water preparations on half-leaves of test plants. Results of these tests are in Table 6. Infectivity of the virus-blood mixtures of tobacco mosaic and potato ringspot viruses were highest at the 1:10 dilution. Only a slight increase in infectivity occurred with dilution up to 1:100 with tobacco ringspot virus.

DISCUSSION.—Results of this investigation showed that such highly infectious viruses as tobacco mosaic, potato ringspot, and tobacco ringspot may be transmitted by insects with chewing mouth parts. The general lack of information concerning the relationships of highly infectious sap-transmissible viruses to insects is understandable. Since such viruses are easy to transmit mechanically, less effort has been made to establish their insect vectors. A greater percentage of the sap-transmissible viruses having vectors are transmitted by insects which lose their infectivity in a relatively short period of time. In this group of vectors are found insects with sucking mouth parts as well as those with chewing mouth parts. Undoubtedly more of the sap-transmissible viruses will be found to be transmitted by chewing insects.

In considering the mode of transmission of the viruses of tobacco mosaic, potato ringspot, or tobacco ringspot by grasshoppers, the most likely assumption is that the viruses are carried mechanically on the insects' mandibles. Markham and Smith (25) have suggested a correlation between the ability of the larva of *Phaedon cochleariae*, vector of turnip yellow mosaic virus, to transmit the virus and the regurgitation of some of the contents of the foregut. A similar phenomenon may occur with grasshoppers and the viruses studied, for the viruses were detected in buccal fluid extracted from grasshoppers several hours after an infection feeding.

The occurrence of local infection away from apparent feeding areas could be assumed to be due to transfer of virus on the feet of grasshoppers. However, grasshoppers may scar or make depressions in leaf tissue with their mandibles while in search of a suitable feeding location; this seems to be the more plausible explanation.

Previous studies show that the juice of macerated insects usually inhibits infectivity of viruses (5, 14, 31). A satisfactory explanation has not appeared for the loss in infectivity of viruses that are easily sap-transmissible when mixed with juices of certain insects. The majority of investigations of this nature have been conducted with juices of macerated insects with little attention directed to the importance of body contents from definite regions of the insect. The effect of buccal fluid and blood of grasshoppers on the in-

TABLE 6.—*Effect of dilution on mixtures each of which contained equal volumes of grasshopper blood and an extract from virus-infected plants*

Virus	Average [a] number of lesions per half-leaf produced with virus-blood mixtures at the following dilutions							
	No dilution		1:10		1:100		1:1000	
	Treated	Control [b]	Treated	Control [b]	Treated	Control [b]	Treated	Control [b]
Tobacco mosaic	99.5	218.0	144.4	181.4	79.0	74.6	11.3	21.4
Potato ringspot	94.5	315.0	213.5	215.9	129.2	137.1	31.7	36.3
Tobacco ringspot	0.3	66.6	3.2	80.1	3.4	0.6	0.0	0.0

[a] Average of 20 half-leaves of *N. glutinosa* for tobacco mosaic, 20 half-leaves of tobacco for potato ringspot virus, and 40 half-leaves of cowpea for tobacco ringspot virus in five tests.
[b] Controls consisted of similar dilutions of virus and water.

fectivity of the viruses studied revealed an inhibitive action, although certain mixtures of virus extracts and buccal fluid or blood did not completely inhibit the infectivity of tobacco mosaic, potato ringspot, or tobacco ringspot viruses. Dilution studies with the 3 viruses and the heat treatments on tobacco mosaic virus indicated that the inhibitive action of buccal fluid was reversible. In dilution studies, extracted blood with virus reacted in a manner similar to buccal fluid with virus, indicating that the inhibitive action of blood was also reversible.

None of the viruses were completely inactivated within the digestive tract of the grasshopper; however, their infectivity was decreased, though only slightly in the case of tobacco mosaic virus. The regurgitated substances present in buccal fluid are composed of secretions passed forward from the mid-gut into the crop and carry with them enzymes present in the mid-gut. Wigglesworth (38) states that the proteinase present in the mid-gut of insects is always of tryptic type. Lojkin and Vinson (24), Caldwell (7), Stanley (35), and Bawden and Pirie (3) have shown that trypsin can inhibit the infectivity of certain plant viruses. Trypsin might aid in the reduction of infectivity of virus in the buccal fluid and naturally voided feces. Failure to obtain tobacco mosaic virus in blood of grasshoppers previously fed on virus-infected plants cannot be explained at this time, unless the intestinal wall acts as a mechanical barrier.

Although the concentration of tobacco mosaic virus was somewhat reduced after being injected into the blood of living grasshoppers, it appears that the blood was a more favorable medium for the virus than buccal fluid. Bennett and Wallace (4) suggested that blood of *Eutettix tenellus* Baker appears to be a favorable medium for preserving curly top virus, for the reaction of the leafhopper blood is slightly alkaline. Since grasshopper blood is only slightly acid, it should not cause any considerable loss of infectivity for tobacco mosaic.

The appearance of tobacco mosaic virus in the buccal fluid after it was injected into the blood of the insects indicates that some virus may pass through the salivary glands. The concentration of virus detected in the buccal fluid was considerably lower than that in blood extracted at the same time. It is possible that only a very small quantity of virus may enter the salivary glands or that conditions in the salivary glands may be somewhat unfavorable for the virus. It is possible that the infection obtained with buccal fluid of grasshoppers whose blood had been injected with tobacco mosaic virus was due to contamination. This appears unlikely since care was taken to prevent entry of the virus into the alimentary tract during injection and to avoid contamination during extraction of the buccal fluid and inoculation of plants.

SUMMARY

The relationships of the differential grasshopper, *Melanoplus differentialis*, to 3 plant viruses were studied. This grasshopper was found to transmit tobacco mosaic, potato ringspot, and tobacco ringspot viruses from tobacco to tobacco. Primary symptoms occurred, in the majority of cases, adjacent to a known feeding area although a very small percentage occurred elsewhere.

Transmission of tobacco mosaic virus by grasshoppers such as might occur in the field was demonstrated. Insects were transferred to healthy tobacco immediately after a 5-min. infection feeding and allowed to feed at will for 17 hr.

Buccal fluid of the grasshopper inhibited the infectivity of plant extracts containing the viruses of tobacco mosaic, potato ringspot, or tobacco ringspot when mixtures of the buccal fluid and virus extracts were inoculated to suitable host plants. Similar results were obtained with mixtures of blood and virus. The buccal fluid decreased the infectivity of the viruses in the following order: tobacco mosaic, potato ringspot, and tobacco ringspot. Blood affected the infectivity of the viruses in the same order.

The inhibitive action of both the buccal fluid and the blood was found to be reversible. The infectivity of certain mixtures of tobacco mosaic virus and buccal fluid was increased by dilution or by heat treatment. Dilution increased the infectivity of mixtures of potato ringspot or tobacco ringspot viruses and buccal fluid. The infectivity of mixtures of viruses of tobacco mosaic, potato ringspot, or tobacco ringspot and blood was increased by dilution.

The inhibitor in the buccal fluid was thermolabile and not readily dialyzable.

Infectivity of the 3 viruses was decreased within the digestive tract of grasshoppers though only slightly in the case of tobacco mosaic virus. Virus was detected in the naturally voided feces of grasshoppers previously fed on plants infected with tobacco mosaic, potato ringspot, or tobacco ringspot viruses. The concentration of tobacco mosaic virus in the feces was comparable to that of a 1:5 dilution of extracts from the plants on which the insects had fed. The infectivity of the potato ringspot virus that passed through the grasshoppers was considerably reduced, and the infectivity of the tobacco ringspot virus was almost completely suppressed.

The viruses of tobacco mosaic, potato ringspot, and tobacco ringspot remained infective for several hours in buccal fluid within grasshoppers. Buccal fluid extracted from grasshoppers showed that tobacco mosaic virus remained infective within the insects for 12 hr., while potato ringspot virus remained infective for 6 hr. and tobacco ringspot virus for 4 hr.

Tobacco mosaic virus was inactivated when injected into the blood of living grasshoppers, however, inactivation was not completed immediately. The blood of 6 of the 12 injected insects remained infective for 10 days, but after 15 days virus was detected in the blood of only 1 insect. After injection of the virus

into the blood, only a very small quantity of virus was detected in the buccal fluid.

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LIFE HISTORY OF THE STEMPHYLIUM THAT CAUSES RED LEAF SPOT OF GLADIOLUS[1]

Ray Nelson and Marie Mooar

Several fungous leaf diseases are economically important on gladiolus in the United States. Massey, in 1916 (5), reported leaf spot caused by *Septoria gladioli*. This disease is distinguished by the occurrence of pycnidia in the lesions. Dimock, in 1940 (1), received severely spotted leaves of gladiolus from Florida and from them cultured a *Botrytis* which he believed to be the causal agent. It is apparent from his description that the leaves were infected by 2 fungi. His statement, "Numerous, small, circular lesions were present on the leaves, about 1 mm. in diameter, light green or yellow in color, with small, reddish dots in the center" is now recognized as describing the leaf spot caused by *Stemphylium* sp. The sunken, brown lesions, 1 mm. wide and 1-4 mm. long on the stems were apparently due to infections by the *Botrytis* which he isolated. Magie, (2, 3, 4) has reported and described the destructive and distinctive Stemphylium and Curvularia leaf spots in Florida. Simmons (7) observed a planting of gladiolus in Canada in 1949 that was severely attacked by *Alternaria fasciculata*. This paper deals with the life history of the gladiolus *Stemphylium* as first reported by the authors in 1949 (6).

HISTORY.—In March, 1938, gladiolus plants were sent to the senior author from Florida for determination of the cause of a leaf blight characterized by lesions that were numerous, small and circular. The fungus isolated from the leaves was not used for infection tests but culture records indicate that it was probably *Stemphylium*. In February, 1945, the senior author collected blighted leaves of several varieties of gladiolus from a 2 acre commercial planting at Encinitas, California. From these leaves *Botrytis* and *Stemphylium* were isolated. Kodachrome transparencies of the plants show that there were 2 types of lesions present; one was small, yellow, circular with red dots in the center, the other larger, irregular, brown with a lighter center. In September, 1946, 2 air mail shipments of gladiolus plants with severely spotted stems and leaves were received from Hayden Preston who had collected them in New York and New Jersey. The lesions were circular, yellow, 1-2 mm. in diameter with prominent red spots in the centers. From the lesions *Stemphylium* was consistently recovered in pure culture. During the same month similar cultures were made from a seedling gladiolus plant growing in the trial ground of the Michigan Gladiolus Society at East Lansing.

Magie, in 1947 (2), recorded the reactions of gladiolus varieties to "leaf spot or blight disease" in his experimental plots in Florida. An accompanying explanation that the list did not distinguish those varieties susceptible to *Botrytis* infection indicates that he was familiar with 2 diseases in the planting. He later (3) reported the isolation of *Stemphylium* and proof of its causal relation to the red leaf spot disease. The senior author collected plants affected with red leaf spot at Portland, Oregon, in September, 1949. From these reports and collections it is apparent that the disease is widely distributed in the United States.

FIG. 1, A. Picardy gladiolus leaves affected with red leaf spot. The lesions are yellow with a red spot in the center.

SYMPTOMS.—The typical lesions of red leaf spot are circular, small—mostly 1-2 mm. in diameter—light green to yellow with a small, prominent red spot in the center (Fig. 1). Two or more lesions may coalesce to form irregularly-shaped spots, usually with a proportional number of red spots in the affected area. Leaves are often so severely spotted that they are killed and under favorable environmental conditions the disease spreads rapidly and destructively.

The most severe outbreaks of red leaf spot occur during or near flowering. Mature plants apparently are more susceptible and abnormally low temperatures

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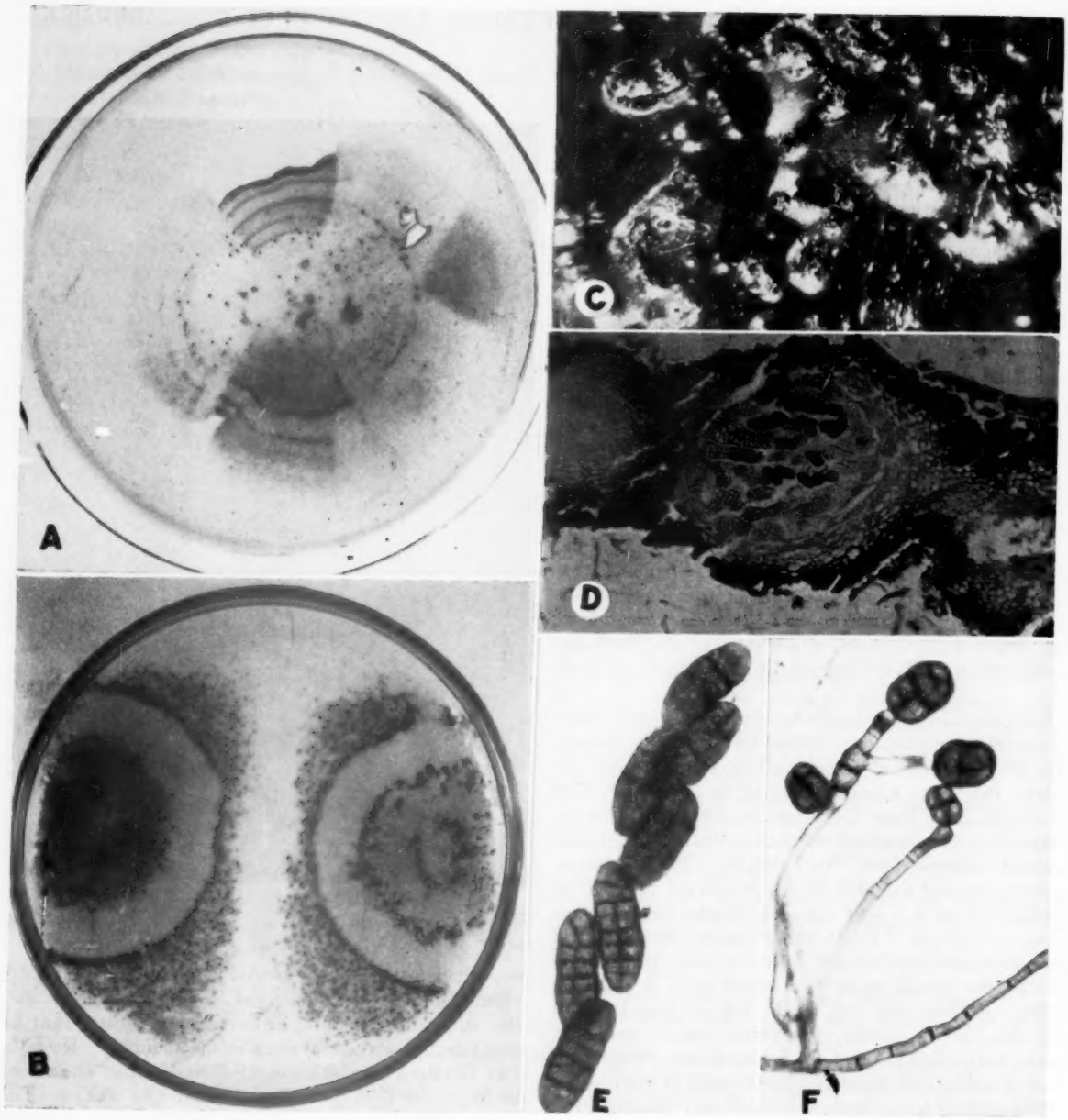

FIG. 2, A. Culture of red leaf spot fungus from a single ascospore seeded on potato agar. Each sector in the thallus originated from one cell of the ascospore; B. Sclerotia in potato agar culture of 2 *Stemphylium* isolates obtained from Picardy gladiolus leaves in September, 1946; C. Stromata with mature perithecia in the original Petri plate culture of *Stemphylium* isolated from a Picardy gladiolus leaf in September, 1946. This culture was held for 19 mo. before the perithecia were mature; D. Section of stroma from prune agar in the original plate culture made in September 1946. Only one of the ascocarps contains asci; E. Ascospores from prune agar culture. They are light tan to dark brown with prominent constriction at each septum; F. *Stemphylium* conidia produced in a culture established from a single ascospore.

just preceding or during flowering appear to condition the plants for the most serious effects of the disease. It is, essentially a warm weather disease as distinguished from Botrytis blight which is most serious in cool, wet seasons. The optimum temperature range for *Stemphylium* in pure culture is 22-28° C., computed with 16-20° C. for *Botrytis*.

THE ASCIGEROUS STAGE.—Fragments of spotted leaves from the shipment of gladiolus plants collected in New York and New Jersey in September, 1946, by Mr. Hayden Preston were washed in running water, rinsed in 95-per cent alcohol and soaked for 30 min. in a 1 per cent solution of Chlorazene. They were then washed for 20 min. in warm, running distilled water

and dried with sterile filter paper. Uncontaminated cultures of *Stemphylium* were recovered from tissue plantings excised from typical lesions. Subcultures were made to potato agar and the original Petri plate cultures were held for further observation. Copious production of sclerotia occurred on the plate cultures (Fig. 2, B) and they were much larger on one plate than those on the others. The Petri plate cultures were subjected to varying environmental conditions. Mostly they were held in an incubator at 20° C. but occasionally they were frozen, thawed, exposed to strong daylight for several days and then returned to the incubator. The agar was repeatedly allowed to dry and subsequently was wetted with sterile prune decoction. Periodic observations revealed the progressive development of ascocarps in the original sclerotial structures. Examination on April 21, 1948, 19 mo. after the plates were seeded, disclosed the presence of mature ascospores (Fig. 2, D). Mature ascocarps developed only on the plates which originally contained the exceptionally large sclerotia (Fig. 2, C, D).

While the stromata were developing they were covered with erect, dark-brown setae which later became procumbent, probably because of the repeated flooding of the plates with the prune decoction. The necks of the ascogones protruded 1-2 mm. above the surfaces of the stromata and drops of moisture accumulated at the tips. No true ostioles were observed. Ascospores were ejected to the lid of the Petri plate and single ascosopore cultures were made on potato agar. Some of the spores were also used for inoculating young leaves and stems of Picardy gladiolus plants.

Stromata were imbedded in paraffin, sectioned, and the sections stained with Delafield's hematoxylin. Progressive stages in the development of the asci and ascospores were observable in the serial sections. The perithecia were not produced singly in the stromata but occurred in groups of 2 or 3 (Fig. 2, D). It is not known whether the multiple development of the perithecia occurs concurrently or as a result of recurrent phases of growth. Mature ascospores, however, were observed in not more than 1 perithecium in a single stroma (Fig. 2, D).

Ascospores were seeded on prune agar in Petri plates and a germ tube was protruded from each cell. The resulting thallus consisted of several distinct sectors varying in color, spore production, number and size of sclerotia, morphology and pathogenesis (Fig. 2, A). Hyphal tips were excised from each sector to establish clonal lines. The lines showed marked cultural differences and variable pathogenicity to Picardy gladiolus plants. No sectoring occurred in the hyphal tip lines indicating the elimination, or a reduction of, the heterokaryosity of the cultures originating from intermingled hyphae from the different cells of the ascospore.

Sclerotial production was markedly reduced after a few transfers of the original cultures and was eventually suppressed in tube cultures. The perithecial stage has not developed in other isolates obtained since 1948 from leaves affected with red leaf spot. Naturally infected leaves bearing numerous red spot lesions were placed on moist soil out of doors in October 1946 and covered with leaves. They were examined for stromata in the spring. A few were observed but ascospores were not seen. Most of the leaves were so badly decayed when examined that stromata could not be identified.

Morphology.—The conidia are mostly echinulate but vary from smooth to echinulate. Some are echinulate, verrucose or even warty only on the upper half of the spore. They are variable in size and shape and the spores from older cultures usually contain more cells and are darker in color than those from young ones. In cultures on potato agar the size of the conidia ranged from 8-14 $\times$ 14-22 μ. In young cultures the spores are nearly cubical, averaging about 11 $\times$ 14 μ. In old cultures they are elongated and average 12 $\times$ 22 μ. The color varies from light tan to dark brown.

Dimensions of the perithecia in the original Petri plate culture were 396-555 $\times$ 460-904 μ, averaging 495 $\times$ 725 μ. The ascospores have 1-5 primary, transverse septa with prominent constrictions at each septum (Fig. 2, E). Their color is similar to that of the conidia. In the original culture on prune agar they were 11-30 $\times$ 29-70 μ. The morphological characters of the ascocarps, asci and ascospores indicate that the fungus belongs in the genus *Pleospora*.

Infection tests.—Infection was readily obtained with conidia removed from red spot lesions on the plants received from New York and New Jersey in 1946 and sprayed on young leaves of Picardy gladiolus plants subsequently held in a saturated atmosphere for 24 hr. The lesions were indistinguishable from those on plants from which the conidia were removed. Ascospores were suspended in water and sprayed on leaves and stems of young plants of Picardy gladiolus. Resulting infections eventuated in typical, circular, yellow spots with red dots in the centers. *Stemphylium* conidia were produced on the leaf lesions and in cultures obtained from the leaf lesions (Fig. 2, F).

These studies have established the relationship between the *Stemphylium* which causes red leaf spot disease of gladiolus and its *Pleospora* ascigerous stage. The specific position of the fungus in the genus has not been determined but it apparently is closely related to *Pleospora herbarum*. *Pleospora herbarum* is a loosely defined species with a wide host range, and infection tests with a variety of these host plants must be made to determine whether the gladiolus is an additional host or whether the fungus is a distinct species.

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PROPOSED AMENDMENT TO THE CONSTITUTION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

BE IT RESOLVED that Article II of the Constitution be amended to read:

"*Section 1.* The Society shall consist of Charter Members, Annual Members, Student Members, Patrons, Sustaining Associates, and Emeritus Members.

"*Section 2.*

(a) *Charter Members.* (No change, as published in PHYTOPATHOLOGY 37: 265.)

(b) *Annual Members.* Any person interested in the study and control of plant diseases shall be eligible for membership. Applications for membership must be endorsed by at least one member of the Society and forwarded to the treasurer with such dues as may be prescribed in the Standing Rules. Applicants may be elected at any regular meeting of the Society or by the Council in the interim.

(c) *Student Members.* Any person interested in the study and control of plant diseases who is enrolled as a senior or graduate student in an accredited college or university is eligible for student membership for a period not to exceed 5 years provided he is not gainfully employed more than ⅔ time. Applications must be endorsed by a member of the Society, and the student and employment status must be certified by the major professor or appropriate department head of the educational institution. Student Members are accorded all the tenure and privileges of Annual Members but must renew the student certification each year when submitting to the treasurer such dues as may be prescribed in the Standing Rules, until they complete their academic training or become employed more than ⅔ time when they automatically become eligible for regular Annual Membership. Applicants may be elected at any regular meeting of the Society or by the Council in the interim.

(d) *Patrons.* (No change, as set forth in Section c of PHYTOPATHOLOGY 37: 265.)

(e) *Sustaining Associates.* (No change, as set forth in Section d of PHYTOPATHOLOGY 3: 265)."

(f) *Emeritus Members* (no change as set forth in Par. 17, Section IIe of PHYTOPATHOLOGY 40: 406).

FURTHERMORE, BE IT RESOLVED that the Standing Rules be amended to read:

"1. *Dues, Arrears, Reinstatements.*

(a) The dues, including subscription to PHYTOPATHOLOGY, for all Annual Members as defined in Article II, Section 2(b) of the Constitution shall be $10.00 a year, and for Student Members, as defined in Section 2(c), shall be $5.00 a year. In order to qualify for the rate as a Student Member, applicants must submit with their dues each year a certificate of student status endorsed by their major professor or the head of the department of instruction.

(b) All dues shall be payable by December 31, and those who fail to make payment to the treasurer by this date shall be considered in arrears and dropped from membership. Members in arrears may be reinstated, without the formality of reelection, by payment of current dues and, in the case of students, certification of student status as specified in Section 2(c) of Article II of the Constitution.

(c) No changes.

(d) No changes.

(e) No changes."

COMMENT

Strong sentiment was expressed at the business meeting in Memphis against imposing heavy membership dues on Student Members of our Society. Everyone was agreed that it would be most unfortunate if students were discouraged from participating in Society affairs and enjoying the advantages of professional contacts until after they had completed their formal studies. At that time, Society finances were not in very good shape and there was no legal recourse except to charge all members the same rate. In the 2 intervening years, Society finances have been placed on a sound basis through the added revenue from members and improved efficiency in the operation of Society affairs. We have been accumulating a surplus with which we expect to expand the services and activities of the Society. We feel that the time is ripe to consider whether students should be accorded special privileges. The Society's Council has, therefore, formulated and approved the above resolution proposing a constitutional change to be voted upon at the second business session at Ithaca, New York, on Wednesday, September 10th. In order to implement this constitutional change, a change in the Standing Rules such as that indicated is also necessary. There is still difference of opinion as to whether the student rate should be ½ that of the senior members or some intermediate amount; the final figure can be decided by a resolution from the floor at the business meeting.

STUDIES ON THE INHERITANCE OF LOOSE SMUT RESISTANCE IN SPRING BARLEYS [1]

Abdur-Rasheed Mohajir, D. C. Arny and H. L. Shands

The loose smut disease of barley caused by *Ustilago nuda* (Jens.) Rostr. has often been a source of loss and trouble to grain growers. Losses are usually small in terms of percentage reduction of crop yield, but may be heavy. The only effective remedy so far recommended is the hot water treatment of infected seeds. This method of control is difficult to use and frequently reduces germination and weakens the plants. Although only a few commercial varieties are resistant to this disease, such varieties of the future offer a more economical means of control. Considerable effort is being made at the Wisconsin Agricultural Experiment Station to breed barley selections with loose smut resistance.

The present investigations were undertaken in an attempt to determine how the reaction to loose smut is inherited in certain varieties and selections. Marker characters were observed to learn their relationship, if any, to loose smut resistance. Favorable linkages, if found, would facilitate the selection of resistant types.

LITERATURE REVIEW.—Shands and Schaller (12) tested 300 barley varieties for reaction to loose smut by artificial inoculation. They found wide varietal differences in reaction to *Ustilago nuda*. Poehlman (4, 5) tested 165 strains of winter barleys with loose smut collections and reported results similar to those of Shands and Schaller.

Seiffert (11), Zeiner (14), Nahmmacher (3), Livingston (2) and Schaller (10) reported on the mode of inheritance of genes for loose smut resistance. Their investigations suggested that the reactions of the varieties studied were determined generally by single pairs of factors.

Adequate disease infection is prerequisite to determinations of genotypes in a study of inheritance of disease reaction. Heavy infection is often insured by using an effective method of inoculation. Shands and Schaller (12) reinvestigated several methods of inoculation, and selected the one whereby dry chlamydospores are introduced into florets by means of a hypodermic needle to which is attached a small rubber bulblet. More recently Cherewick and Popp (1) improved Moore's partial vacuum method to increase the ease and rate of inoculation of barley florets, but did not increase percentages or uniformity of infections.

Another method that possibly could be used for identifying genotypes in inheritance studies is that described by Russell (9). He suggested that the embryo test for infection was valuable as a routine smut estimation especially in connection with seed evaluation. He found good agreement between the embryo infections and smut infected plants. Popp (6) in barley experiments found that the correlation between the embryo infection and smutted plants was positive and highly significant, thus substantiating Russell's conclusion.

MATERIALS AND METHODS.—The needle method of smut inoculation described by Shands and Schaller (12) and later by Schaller (10) was used in these investigations. Inoculum used was a composite of spores from susceptible parents and lines. As the inoculum did not infect Trebi or selections with resistance from this variety, the Trebi race of *U. nuda* was not present.

Parent varieties and selections with their origin are listed below:

Parent	*Place of origin or parents*
Abyssinia (C.I. 668)	From Abyssinia
Arthritis (C.I. 6174)	Maison Carré × Manchuria
Jet (C.I. 967)	From Abyssinia
Valentine (C.I. 7242)	Trebi × Chevron
C.I. 1413	From Australia
C.I. 5324	Composite cross selection
H46-4-4-6-7	Chevron × Brachytic
H47-28	Trebi × Chevron
X191-24	Barbless × Newal
X217-57-4	" × Trebi
X237-3	" × Dorsett
X238-3	(Oderbrucker [2] × Lion) × Dorsett
X255-1	(Barbless × Olli) × (Barbless × Chevron)

These varieties were used as part of a larger barley improvement program in progress at the Wisconsin Agricultural Experiment Station. The first 3 varieties listed above and C.I.'s 1413 and 5324 were included primarily because of having loose smut resistance. Valentine and H47-28 were used because of the favorable linkage of stem rust and loose smut resisting genes. The H46 selection was resistant to both diseases. The remaining selections with X numbers were six-rowed, smooth-awned, and white.

Parents showing more than 50 per cent loose smut were classified as susceptible, those with 26 to 50 per cent as moderately susceptible, those having 11 to 25 per cent as moderately resistant and those which had 0 to 10 per cent as resistant.

Tests on the hybrid material were made largely in the F_3, and to a limited extent in the F_1, F_2, F_4 and backcross generations. F_1 plants were tested by inoculating the crossed florets 24 hrs. after pollination. Because results of F_1 and F_2 tests were difficult to interpret, florets of individual F_2 plants were inoculated and genotypes established by means of smut percentages obtained in the F_3 generation. Florets of 3 spikes of each F_2 plant were inoculated in susceptible × resistant crosses and 2 spikes each in moderately susceptible × resistant and resistant × resistant crosses. Plants grown from inoculated spikes of each F_2 plant constituted an F_3 line. Seed from a noninoculated spike of each F_2 plant was grown to verify the classification of marker characters in the F_3 generation. The

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TABLE 1.—*Loose smut reaction of F_1 and F_2 generations of various barley crosses and their reciprocals when inoculated with Ustilago nuda*

Cross	Generation	Tests[a]	Range of infection	Total plants	Weighted Average
		No.	Per cent	No.	Per cent
X255-1 × Abyssinian	F_1	2	0–0	23	0.0
Abyssinian × X255-1	F_1	2	0–0	14	0.0
X255-1 × Abyssinian	F_2	11	0–17	184	7.6
X191-24 × X237-3	F_1	3	0–0	19	0.0
X237-3 × X191-24	F_1	2	0–0	16	0.0
X191-24 × X237-3	F_2	10	0–36	145	12.4
Arthritis × Jet	F_1	2	0–0	14	0.0
Arthritis × Jet	F_2	7	0–14	110	4.5
Arthritis × Abyssinian	F_1	1	0–0	18	0.0
Arthritis × Abyssinian	F_2	10	0–15	152	7.8
C.I. 1413 × X217-57-4	F_1	3	0–0	28	0.0
C.I. 1413 × X217-57-4	F_2	9	0–21	145	11.1

[a] Each test represents 1 inoculated head.

material in the backcross was handled in a similar manner. The parent material was inoculated at the same time as the progenies of their crosses. For verifying genotype assignments, limited tests were made on the F_4 generation by inoculating selected F_3 lines having different smut percentages.

EXPERIMENTAL RESULTS.—The smut percentages obtained in the F_1 and F_2 generations of 5 crosses and 2 reciprocals are in Table 1. The loose smut reactions of 13 barley varieties and selections and the hybrid populations of the F_3 and F_2 backcross progenies are in Table 2 (opposite page).

Susceptible × resistant.—a) *X255-1 × Abyssinian.*—Abyssinian is very resistant, and X255-1 is susceptible when artificially inoculated with *Ustilago nuda.*

In the F_1 generation no infection occurred in reciprocal crosses indicating that the resistance was dominant (Table 1). The 7.6 per cent infection in the F_2 again indicated that resistance was dominant.

The distribution of F_3 lines into 5 per cent classes is in Table 2. The average infection of all lines was 12.3 and 15.7 per cent respectively for the 2 years. The infection range of the X255-1 parent suggested that lines above 40 per cent were susceptible. The proportion of lines in the 2 groups, 0-40 and 41-100, indicated a 2-factor mode of inheritance with resistance being dominant. With homozygous resistant and heterozygous lines in the first group, and homozygous susceptible in the second, a 15:1 ratio is suggested, indicating that the resistance of Abyssinian was controlled by 2 factor pairs. Chi-square tests on the basis of 2-factor hypothesis gave P values between .50 and .20, indicating a satisfactory agreement between the calculated and observed ratios.

Several lines from the F_3 populations of 1949 were further tested in the F_4 generation (Table 3). Two heads were inoculated on each of 2-4 F_3 plants in each line. Ten lines having no infection in the F_3 remained free from smut in the F_4, but lines in the 1 to 40 per cent classes showed considerable heterozygosity. All lines with more than 40 per cent smut remained susceptible. These results substantiate the separation made in the F_3.

b) *F_1(X255-1 × Abyssinian) × X255-1.*—Florets produced on the backcross plants were inoculated. For the F_2 backcross lines the smut distribution (Table 2) was based on the average infection of 3 replications. On the same basis as the F_3 classification of resistant and heterozygous lines, the F_2 backcross lines were divided at the 40 per cent level of infection. There were 38 lines in the 0 to 40 per cent classes, and 14 lines in the 41 to 65 per cent classes, which suggest a 3:1 ratio. In a backcross this could be expected on the basis of a 2-factor hypothesis when the heterozygous classes are indistinguishable. The additional complement of susceptible factors did not increase the average infection so much as might have been expected.

TABLE 3.—*Infection in F_3 lines compared with infection in F_4 families obtained by inoculating random plants in non-infected F_3 populations. X255-1 × Abyssinian*[a]

F_2 plant number	Average infection in F_3[b]	F_4 generation		
		Lines tested	Infection	
			Average	Range
	Per cent	No.	Per cent	Per cent
180	2	2	0	0–0
281	2	3	12	0–35
131	4	2	4	0–7
310	11	3	42	20–69
123	22	2	12	11–12
278	23	2	24	0–47
102	24	2	12	0–23
323	25	2	22	0–44
73	36	2	48	21–75
307	38	2	82	78–86
81	40	4	46	30–75
6	47	2	45	40–50

[a] 10 lines with no smut in the F_3 had no smut in the F_4 test.
[b] From 1949 population.

c) *X191-24 × 237-3.*—X191-24 is susceptible and X237-3 is highly resistant to loose smut. The reactions of the F_1 and F_2 generations of X191-24 × X237-3 and their reciprocal crosses are in Table 1. The absence of infection in F_1, and the infection of 12.4 per cent in the F_2 generation indicates that the resistance of X237-3 was dominant.

The distribution of F_3 lines is in Table 2. The average infection of all lines was 20 per cent. Several lines appeared to be more susceptible than X191-24 indicating that some transgressive segregation occurred. There is a low point in the distribution of F_3 and backcross lines near the 35 per cent infection level. The infection of the susceptible parent usually exceeded 35 per cent. Therefore it appears logical to classify lines with this amount of infection or more as being susceptible, and those lines with less infection as being heterozygous, or resistant to smut. The proportion of the 2 groups suggests a rather simple mode of inheritance with resistance being dominant. In the 2 years' results there were 208 lines in the 0 to 35 per cent classes and 49 in the 36 to 90 per cent infection

TABLE 2.—*Distribution of parent rows[a] and F_2 and F_3 backcross progenies for loose smut infection by percentage classes for crosses indicated*

Parent or Cross[a]	Year	Loose smut percentage classes: 0	1–5	6–10	11–15	16–20	21–25	26–30	31–35	36–40	41–45	46–50	51–60	61–70	71–80	81–100	Total	Average infection
																	No.	Per cent
X255-1 × Abyssinian	1949	38	9	3	3	5	12	5	2	3	0	1	0	0	1	0	82	12.3
	1950	71	20	10	12	8	12	11	1	3	1	3	7	1	3	1	164	15.7
F_1(X255-1 × Abyssinian) × X255-1	1950	18	0	3	4	3	5	0	1	4	0	2	8	4	0	0	52	16.4
X255-1	1949	0	0	0	0	0	0	0	0	0	0	0	2	4	3	1	10	70.0
	1950	0	0	0	0	0	0	0	0	0	1	0	1	2	5	1	10	68.6
X191-24 × X237-3	1949	12	5	7	5	1	4	5	6	1	3	2	3	3	0	0	57	20.8
	1950	30	22	34	23	21	21	9	6	7	4	6	8	4	2	3	200	20.5
F_1(X191-24 × X237-3) × X191-24	1950	8	2	2	2	3	1	2	0	1	2	3	0	3	0	0	29	21.5
X191-24	1949	0	0	0	0	0	0	0	1	0	0	3	6	0	0	0	10	51.5
	1950	0	0	0	0	0	0	0	1	0	2	0	5	0	0	0	8	50.2
Arthritis × Jet	1949	27	3	11	1	0	1	1	0	1	0	0	0	0	0	0	45	6.9
	1950	61	1	9	7	6	1	1	0	1	0	0	0	0	0	0	87	4.7
Arthritis	1949	0	0	0	0	0	0	0	1	4	2	3	0	0	0	0	10	46.2
	1950	0	0	0	0	0	1	1	2	3	2	1	0	0	0	0	10	36.5
Arthritis × Abyssinian	1949	37	4	4	1	1	0	1	1	0	0	1	1	0	0	0	51	7.3
	1950	71	2	4	6	4	3	1	2	0	0	0	0	0	0	0	93	4.0
C.I. 1413 × X217-57-4	1949	20	5	0	0	2	2	1	0	0	0	0	0	0	0	0	30	5.2
	1950	30	11	14	1	8	1	2	0	1	0	3	0	0	0	0	71	9.8
C.I. 1413	1949	1	0	2	2	1	0	3	0	1	0	3	0	0	0	0	10	17.1
	1950	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0	3	24.6
X217-57-4	1949	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10	0.0
	1950	1	6	3	0	0	0	0	0	0	0	0	0	0	0	0	10	5.6
X238-3 × H46-4-4-6-7	1949	18	2	2	1	2	0	1	0	0	0	0	0	0	0	0	26	4.0
	1950	26	14	24	7	1	0	1	0	0	0	0	0	0	0	0	73	6.4
X238-3	1949	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10	0.0
	1950	1	0	3	2	0	0	0	0	0	0	0	0	0	0	0	6	10.6
H46-4-4-6-7	1949	6	1	2	1	0	0	0	0	0	0	0	0	0	0	0	10	3.5
	1950	1	7	2	0	0	0	0	0	0	0	0	0	0	0	0	10	4.6
Abyssinian × Valentine	1950	93	1	5	3	0	0	0	0	0	0	0	0	0	0	0	102	0.6
C.I. 5324 × H47-28	1949	28	8	5	2	0	0	1	0	0	0	0	0	0	0	0	44	2.5
	1950	68	2	6	3	2	0	2	0	0	0	0	0	0	0	0	83	3.5
H47-28	1949	5	2	2	0	1	0	0	0	0	0	0	0	0	0	0	10	4.5
	1950	2	3	4	0	0	0	0	0	0	0	0	0	0	0	0	9	5.5

[a] No infection occurred in 2 years' tests involving about 20 replications of Abyssinian, X237-3, Jet, Valentine, and C.I. 5324.

classes. This fits a 13:3 ratio indicating the action of 2 factors, 1 dominant and 1 recessive. The dominant factor comes from X237-3 while the recessive factor for resistance might come from X191-24 as this selection apparently is not completely susceptible. There were some F_3 lines with infections that exceeded those of X191-24.

Several lines from the 1949 F_3 population were tested in the F_4 generation (Table 4). Of 5 lines which had no infection in the F_3 generation 3 bred true and 2 segregated in the F_4. Lines with 1 to 33 per cent infection in the F_3 were generally intermediate in their F_4 reaction.

Those with 35 to 63 per cent in the F_3 showed the same range in the F_4 as the susceptible parent. The tests on the F_4 generation indicate that the initial

TABLE 4.—*Infection in F_3 lines compared with infection in F_4 families obtained by inoculating random plants in non-infected F_2 populations. X191-24 × X237-3* [a]

F_2 plant number	Average infection in F_3 [b]	F_4 generation		
		Lines tested	Infection	
			Average	Range
	Per cent	No.	Per cent	Per cent
473	0	3	4	0–6
489	0	3	13	0–28
461	2	3	0	0–0
455	4	3	0	0–0
398	4	4	7	5–10
450	5	3	5	0–10
510	8	3	11	0–20
439	9	5	10	0–17
395	9	3	29	25–36
518	13	4	6	0–8
364	22	3	21	17–23
387	26	3	25	23–27
538	28	3	30	29–30
409	33	4	26	24–29
412	36	4	46	30–61
360	44	3	31	21–50
417	45	3	25	23–25
391	49	3	56	48–70
356	49	3	51	40–63
447	63	3	69	57–75

[a] 3 lines with no smut in the F_3 had no smut in the F_4 test.
[b] From 1949 population.

division point at the 31 to 35 per cent level was essentially correct.

d) *F_1(X191-24 × X237-3) × X191-24.*—The smut tests were made also on the F_2 backcross lines. The distribution of 29 lines is indicated in Table 2. The small number of lines involved here may not give a reliable estimate of the distribution but there is some indication of 2 class groups; 0 to 35 and 36 to 70 per cent. Twenty lines in the 0 to 35 per cent group and 9 lines in the 36 to 70 group constitute approximately ¾ and ¼ of the population respectively (P lies between .50 and .20). This ratio would be expected if 2 factor pairs were involved, with resistant classes being indistinguishable under the conditions of this test.

TABLE 5.—*Infection in F_3 lines compared with infection in in F_3 families obtained by inoculating random plants in non-infected F_2 populations, F_1(X191-24 × X237-3) × X191-24*

Backcross plant number	Average infection in F_2	F_3 generation [a]		
		Lines tested	Infection	
			Average	Range
	Per cent	No.	Per cent	Per cent
2489	4	3	19	16–22
2468	9	7	27	5–52
2401	16	7	19	10–33
2460	16	7	24	0–37
2464	25	7	33	3–42
2408	27	5	30	0–39
2418	43	7	49	21–57
2440	65	7	51	43–61

[a] F_3 generation tested in the greenhouse.

Eight F_3 backcross lines were further tested by growing 2 heads each from 3 to 7 plants per line in the greenhouse (Table 5). Lines below 35 per cent in the F_2 appeared to be intermediate in the F_3 while lines above 35 per cent remained susceptible. Unfortunately lines with 0 per cent infection in the F_2 were not tested further.

Moderately susceptible × resistant.—a) *Arthritis × Jet.*—Arthritis was found to be moderately susceptible to loose smut whereas Jet showed,no infection in all tests. The absence of infection in F_1 and low amount of infection in the F_2 generation suggested that the resistance of Jet was dominant (Table 1).

The F_3 distribution as given in Table 2 suggests 3 smut reaction groups. The first group is comprised of lines having 0 to 5 per cent infection which may be classed as "resistant." The second group includes lines with 6 to 20 per cent infection. The separation at the 20 per cent level was indicated by the infection percentages in Arthritis. The third group is comprised of lines falling in the infection classes of 21 to 40 per cent. The distribution of F_3 lines suggested a ratio of 11:4:1 assuming 2 factors both of which are dominant and carry equal weight in conditioning loose smut resistance. The resistant class would be made up of lines homozygous dominant for at least 1 factor or heterozygous for both; the intermediate class of lines homozygous recessive for 1 factor and heterozygous for the other; and the susceptible class of lines homozygous recessive for both factors. The chi-square indicated a satisfactory fit giving a P value of .95 to .50 for each year and for both years combined.

A few of the F_3 lines were tested further in the F_4 generation (Table 6). The results indicated that 18 lines which were free of smut in F_3 remained free in the F_4 whereas lines having 1 to 5 per cent gave similar percentages in the next generation. Lines with 6 to 20 per cent smut gave similar percentages in the F_4 and may be considered heterozygous. Lines having more than 21 per cent smut remained moderately susceptible.

TABLE 6.—*Infection in F_2 lines compared with infection in F_4 of families obtained by inoculating random plants in non-infected F_2 populations, Arthritis × Jet* [a]

F_2 plant number	Average infection in F_2	F_4 generation		
		Lines tested	Infection	
			Average	Range
	Per cent	No.	Per cent	Per cent
1314	3	5	9	0–15
1282	4	4	5	0–10
1269	4	3	4	0–5
1336	10	3	15	0–20
1343	10	3	19	10–20
1354	14	3	24	20–30
1293	31	3	32	20–50
1262	41	5	30	20–40

[a] 18 lines with no smut in the F_3 had no smut in the F_4 test.

TABLE 7.—*Infection in F_3 lines compared with infection in F_4 families obtained by inoculating random plants in non-infected F_2 populations. Arthritis × Abyssinian* [a]

F_2 plant number	Average infection in F_3	F_4 generation		
		Lines tested	Infection	
			Average	Range
	Per cent	No.	Per cent	Per cent
1417	2	3	8	0–10
1411	3	4	6	0–10
1435	6	5	18	10–25
1385	9	4	12	0–15
1452	16	5	13	10–20
1474	35	4	40	35–45
1511	47	3	51	45–56

[a] 4 lines with no smut in the F_3 had no smut in the F_4 tests.

Although Schaller (10) reported that Jet has 1 factor pair for resistance, the present work indicated that 2 pairs are involved. Each factor pair in combination with the Arthritis factors exerts a strong influence and can prevent smut infection.

b) *Arthritis × Abyssinian.*—The absence of infection in the F_1 and the low infection of 7.8 per cent in the F_2 generation indicated that the resistance of Abyssinian was dominant (Table 1).

The distribution for loose smut infection of the F_3 lines (Table 2) was divided into 3 groups as in the previous cross, Arthritis × Jet. The chi-squares indicate a satisfactory fit with the expected 11:4:1 ratio when the 2 years are considered separately giving P values of .10 to .05 and .20 to .10 respectively, but when the 2 years were combined a P value between .05 and .02 was obtained.

The smut reaction of selected lines in the F_4 generation is shown in Table 7. The 4 lines which had no infection in the F_3 generation bred true. Lines with 1 to 20 per cent were slightly more susceptible in the F_4. These lines probably segregated for only 1 of the 2 factor pairs with the other pair being homozygous recessive. The homozygous moderately susceptible lines had approximately the same infection in both generations.

Moderately resistant × resistant.—a) *C.I. 1413 × X217-57-4.*—The data in Table 2 indicate that C.I. 1413 was moderately resistant and X217-57-4 resistant. The absence of infection in the F_1 and the infection of 11.1 per cent in the F_2 population (Table 1) support the assumption that resistance of X217-57-4 was dominant and that the resistant gene functioning was probably from Trebi. The reaction of C.I. 1413 was so variable as to make a plausible factorial analysis impossible, although a 1-factor difference is suggested. Two lines gave infections above 10 per cent in both F_3 and F_4 generations.

Resistant × resistant.—An attempt was made to determine the relationship of factors carried by different resistant varieties. The distribution of F_3 lines is in Table 2. Only the F_3 and F_4 generations were tested for loose smut reaction.

a) *X238-3 × H46-4-4-6-7.*—The loose smut infection in both parents indicated that a slightly lower type of resistance was involved than in Jet and Abyssinian. The source of resistance in both these parents was of considerable importance in the study of this cross. X238-3 derived its resistance from Dorsett which was shown by Schaller (10) to have a single factor pair for moderate resistance. H46-4-4-6-7 contained in its parentage Brachytic which was also found by Schaller to have a single factor pair for resistance. The distribution of F_3 lines in the present investigation indicated the presence of 2-factor pairs.

b) *Abyssinian × Valentine.*—The presence of a few smutted lines in the 1 to 15 per cent classes did not necessarily imply segregation. The Valentine gene may have been recovered, giving essentially similar results as other tests where Valentine had some smutted plants. The average infection of 0.6 per cent in F_3 lines further supported this assumption. These varieties probably have 1 factor in common for resistance to loose smut.

c) *C.I.5324 × H47-28.*—Although there was an indication of transgressive segregation in the F_3 generation, no segregation was noted in the F_4 generation. The behavior of this cross suggested that the 2 varieties have at least 1 factor pair in common for loose smut resistance.

Association of characters.—A summary of the crosses (Table 8) indicates that there was a suggestion of association between the 2-rowed character of Abyssinian and Jet with loose smut resistance. The chi-square tests showed independence of row number and loose smut reaction, but association was suggested because of higher average infection in 6-rowed than in 2-rowed genotypes. Possibly the large kernels of 2-rowed varieties may retard infection more frequently than the smaller kernels of 6-rowed varieties. No direct evidence was obtained on this point. All the other marker characters were found to be independent of loose smut reaction. Smut infections were so low in the cross C.I. 5324 × H47-28 that the indicated association is of doubtful value, although it does agree with the findings of Shands (13).

The non-parasitic leaf spot reaction in Abyssinian was found to be dominant and conditioned by a single factor pair. The leaf spot reaction appeared to be independent of loose smut reaction.

DISCUSSION AND SUMMARY

In a series of crosses involving varieties with differences in smut reaction, factorial analyses were made concerning the mode of inheritance of loose smut reaction.

Loose smut reaction of F_1, F_2 and F_3 generations of the crosses of susceptible × resistant varieties indicated that the resistance was dominant over susceptibility. Reciprocals of 2 crosses in the F_1 generation also indicated that the resistance was dominant.

The smut resistance of Abyssinian and Jet were

TABLE 8.—*Summary of genes and linkage groups studied in various crosses with reference to their loose smut reaction*

Cross	Loose smut factor differences between parents	Factor designation	Associations [a] Independent of	Associated with
X255-1 × Abyssinian	2	.....	V^tv(I), Rr(V), Tt(VII),	V^tv ? [b]
X191-24 × X237-3	1	Un_4 (Dorsett gene)	Npnp[c] (Misc.)	.....
			E_2e_2 (Misc.)	Vv [b]
Arthritis × Jet	2	Un_3 (Jet gene) + 1 additional gene	Vv(I), Bb(II), Nn(III)	
Arthritis × Abyssinian	2	.....	Npnp (Misc.)	V^tv
C.I. 1413 × X217-57-4	1	*Un* (Trebi gene)	Bb(II), Rr(V)	.....
X238-3 × H46-4-4-6-7	At least 1 in common	Un_4 (Dorsett gene)	Rr(V), Ss(V), Tt(VII)	.....
Abyssinian × Valentine	do.	.....	V^tv(I)	.....
C.I. 5324 × H47-28	do.	.....	E_2e_2 (Misc.), Ss(V)	Tt

[a] Symbols from Robertson et al. (7 and 8). Roman figure in parentheses indicates linkage group.

V—Two-rowed spikes, distichon type	v—Six-rowed spikes
V^t—Two rowed spikes, deficiens type	
B—Black lemma and pericarp	b—White lemma and pericarp
N—Hulled caryops.s	n—Naked caryopsis
R—Rough awns	r—Smooth awns
S—Long-haired rachilla	s—Short-haired rachilla
T—Stem rust resistant	t—Stem rust susceptible
E_2—Long glume awns	e_2—Short glume awns
Np—Non parasitic leaf spots	np—No spots

[b] Chi-square tests showed independence of row number with loose smut reaction; however, greater infection in six-rowed than in 2-rowed genotypes suggested a possible association between row number and smut reaction.

[c] Suggested symbol only, subject to the approval by barley genetics committee.

found to be conditioned by 2 factor pairs in each variety when crossed to the susceptible and moderately susceptible varieties, X255-1 and Arthritis. The low amount of infection in the F_2 generations supported the hypothesis that there were more than 1 factor pairs present. There was indication of the existence of a gene in the Jet variety in addition to the 1 reported by Schaller (10). The indication of this additional gene may have been caused by the moderately susceptible Arthritis parent being different from the highly susceptible Colsess parent used by Schaller.

An indication of transgressive segregation was noted in X191-24 × X237-3. X237-3 was considered to have 1 factor pair for resistance. This selection derived its resistance from Dorsett which was reported by Schaller (10) as having a single gene conditioning the smut resistance. The transgressive segregation can be explained by a recessive factor from X191-24.

For smut reaction in the cross Arthritis × Jet and Arthritis × Abyssinian the resistance appeared to be conditioned by 2 factor pairs. A 11:4:1 ratio was suggested in both crosses indicating that the 2 factors present in Abyssinian and the 2 in Jet impart a strong resistance to loose smut to a high proportion of the progenies. Further, the smut infection levels were generally low, showing that Arthritis added a measure of resistance when compared to a fully susceptible variety.

In the moderately resistant × resistant cross, C.I. 1413 × X217-57-4, a plausible factorial analysis could not be made because of the variability of the C.I. 1413 parent.

In the resistant × resistant cross, C.I. 5324 × H47-28, no segregation was noted in the F_4 generation but there was indication of slight transgressive segregation in the F_3 generation. The behavior of this cross suggested that the 2 varieties have at least 1 factor in common.

In X238-3 × H46-4-4-6-7 there was a slight indication of transgressive segregation in F_3 progenies but the F_4 generation gave no reliable segregation different from what could be expected in the parents. X238-3 has in its parentage Dorsett and X173-$_1$0-5-6-1. Brachytic is the main source of resistance in H46-4-4-6-7. The Dorsett factor was found by Schaller (10) to be independent of the Brachytic gene and of the weak X173 gene. The present data did not provide enough evidence to determine the relationship between the factors of the above parents. There was a suggestion of transgressive segregation in the F_3 distribution.

Some F_3 lines in the cross Abyssinian × Valentine were as resistant as Abyssinian while others were similar to Valentine for smut reaction. In previous work Valentine was not as completely resistant to loose smut as Abyssinian. The presence of few lines with low smut percentages probably indicated the recovery of Valentine type. These varieties may have at least 1 factor in common but it could not be determined from this cross whether or not there are additional genes in Abyssinian.

There was a suggestion of association of loose smut reaction with spike row character in crosses where Abyssinian or Jet were involved. The evidence is not conclusive but it seems that at least 1 of the Abyssinian and Jet genes might be in the linkage group I. The location of the other gene or genes in these varieties was not determined.

The symbols for loose smut factors recommended by Schaller (10) are as follows: *Un* for Trebi, Un_2 for Missouri Early Beardless, Un_3 for Jet, Un_4 for Dorsett,

Un$_5$ for X173-$_1$0-5-6-1. It seems that Abyssinian carries at least 1 gene that is different from the Trebi gene since Abyssinian is resistant to both common *U. nuda* and the Trebi race of *U. nuda* (unpublished data from R. G. Shands). Since Schaller found no association between the Jet gene and any of the other marker characters he studied, the indicated association between the 2-rowed character and smut resistance of Abyssinian found in the present work suggests that there is some difference between the genes for smut resistance in Abyssinian and Jet. The inoculated progeny of the cross Abyssinian and Jet, not studied in the present work, might add more analytical information. Until the cross Abyssinian × Jet is studied a definite gene symbol probably should not be assigned.

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TRANSIT ROT OF VANDA ORCHID BLOSSOMS [1]

William A. Feder [2]

The past few years have witnessed an enormous increase in the number of Vanda Miss Joaquim orchid blossoms shipped to mainland flower markets from the Hawaiian Islands. This portion of the Island orchid industry has become a contributing factor in the economy of the Hawaiian Islands. During the peak of the 1949-50 and 1950-51 winter season a severe outbreak of blossom rotting was reported and losses amounting to thousands of dollars were sustained.

Transit rot of Vanda orchid blossoms is caused by the action of a fungal complex composed of species of *Botrytis*, *Gloeosporium*, and *Alternaria*. The disease symptoms vary with the makeup of the complex, usually assuming the symptom picture of the predominant fungus, which in turn is determined apparently by the locale in which the flowers are produced.

Other writers (1) have briefly described the *Gloeosporium* phase of this problem but, with the exception of Hendrix and Murakishi (5), have failed to describe the entire fungal complex involved.

A previous brief report by the writer (2) is amplified by the present paper which describes the separation and identification of the components of the fungal complex and demonstrates the separate and combined effects of these fungi upon Vanda blossoms in transit.

SYMPTOMS AND SIGNS.—The symptoms of transit rot are peculiar in that they seldom show in the field. Instead they become visible after the blossoms have been packed and are in transit. The first symptoms appear about 3 to 5 days after packing, the exact time depending upon the temperature at which the flowers are shipped. The picked flowers are packed in cardboard containers, in which are placed damp cotton pads, or some similar humidifying agent. The flower boxes are then sealed in various ways and shipped by air to mainland destinations. After 3 to 5 days, small irregular-shaped chocolate-brown spots become apparent on petals and sepals, usually 1 spot on a single flower part (Fig. 1). The spots enlarge and spread, involving the entire blossom. Instead of spots there may be marginal browning, where the petal contacts the moist cotton pad. The flowers are closely packed and the rotting soon spreads from flower to flower, until all the blossoms in a pack are affected. Once an entire blossom becomes involved, it rapidly loses its turgidity, becomes first limp, then soggy, and finally breaks down into a mushy, brown, rotten mass (Fig. 2). If the rotted blossoms are retained beyond this

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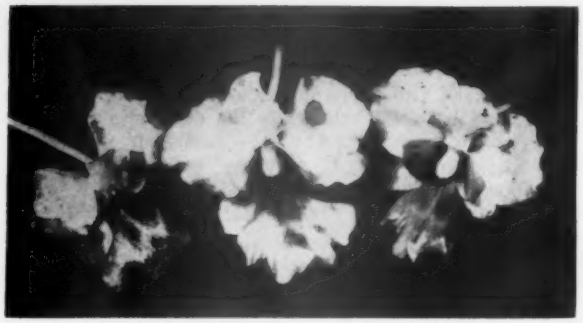

FIG. 1. Vanda Miss Agnes Joaquim blossoms showing symptoms of Transit rot. Blossoms inoculated from left to right with single spore cultures of *Botrytis*, *Gloeosporium*, and *Alternaria* respectively.

point they soon become covered with a mat of white-grey mycelium. The erect conidiophores of *Botrytis* appear scattered over the blossom surface and excessive sporulation takes place. This entire sequence of events takes place in 8 to 14 days, depending upon environmental conditions. Somewhat later, the darker gray mycelium and the fruiting bodies of a *Gloeosporium* species may also appear, as may the dark spore bodies of a species of *Alternaria*.

The *Gloeosporium* and *Alternaria* species cause visible field symptoms, and therefore blossoms thus infected are usually culled before they reach the packing box. However, both these fungi are capable of causing blossom rots which are often difficult to distinguish from *Botrytis* rot, once the blossom has reached the last stages of disintegration. Further, all 3 fungi may act together to form a rot complex.

Blossoms infected with *Gloeosporium* become marked by pale white translucent spots, which develop concentrically, with entire margins (Fig. 3). These spots turn brown and enlarge until whole flower parts become involved. This phase of the disease may be evident in the field in contrast to the early *Botrytis* symptoms which are visible only after the flowers have been packed and shipped. *Gloeosporium* rot progresses much the same as *Botrytis* rot, eventually causing a watery breakdown of the blossom. The watery, rotten flowers resulting from an attack of *Gloeosporium* are a darker brown than those resulting from the ravages of *Botrytis;* this characteristic, plus the fact that the mycelium of *Gloeosporium* is a darker gray than that of *Botrytis*, is all that distinguishes the 2 diseases in the pack.

FIG. 2. Vanda blossoms badly infected with *Botrytis*. These blossoms are pictured about 10 days after inoculation with a single spore culture of *Botrytis*. They are partially rotted and covered with mycelium.

FIG. 3. Vanda blossoms showing effects of a natural infection of *Gloeosporium*. This type of spotting occurs in the field during periods of rainy, cloudy weather.

Field infections of Vanda blossoms with *Alternaria* sp. are readily discernable by the pock-marked appearance of flowers (Fig. 1). The pock marks are tiny brown flecks which usually appear on older flowers or on the damaged portions of younger blooms. These symptoms are readily visible, enabling most flowers so infected to be culled. However, if a diseased flower is inadvertantly packed among healthy ones, the disease will eventually spread to the healthy flowers. The brown specks enlarge until they involve the entire flower. The flower then turns brown and dries out, in contrast to the watery end-products resulting from infections of the other 2 fungi. *Alternaria* sporulates profusely after about 2 wk. in the pack.

CAUSE.—Single spore isolations made from diseased blossoms consistently yielded 3 genera of fungi, *Botrytis*, *Gloeosporium*, and 3 different isolates of *Alternaria*. All fungi were cultured on potato-dextrose agar or broth and active sporulation occurred on both media. These fungi were not classified as to species.

Spore suspensions of these fungi, made in 100 cc. of sterile water, were sprayed on healthy Vanda Joaquim flowers grown in a disease-free area. Other healthy

TABLE 1.—*The effect of different temperatures on disease development of Vanda blossoms inoculated with Botrytis, Gloeosporium, and Alternaria*

Name of Fungus	Days after Inoculation	Percentage of blossoms infected			
		12°C.	16°C.	20°C.	24°C.
Botrytis	5	100	100	100	100
	12	100	100	100	100
Gloeosporium	5	10	20	100	100
	12	50	100	100	100
Alternaria	5	20	0	30	50
	12	40	?	30	100
Control	5	0	0	0	0
	12				

flowers from the same area were sprayed with sterile water and used as controls. All flowers were packed in large sterilized aluminum baking trays which were lined with moistened paper toweling and sealed with Pliofilm. Each tray held 24 blossoms; 32 trays were prepared: 8 containing blossoms sprayed with *Botrytis*, 8 sprayed with *Gloeosporium*, and 8 sprayed with a mixture of *Alternaria* isolates. Eight more sprayed with sterile water served as controls.

In order to study the infective temperature range of the isolates, 2 trays of each type were placed at each of 4 temperatures; 12°C., 16°C., 20°C., and 24°C. and all trays were incubated for 14 days. Observations made after 5 and 12 days incubation are listed in Table 1.

Each fungus produced symptoms peculiar to itself but as incubation continued these symptoms became almost indistinguishable. All the fungi were capable of producing transit rot over the entire temperature range tested, but each fungus appeared to have an optimum infective temperature range. *Botrytis* was more active at the lower temperatures, the other 2 organisms at the higher temperatures. The controls remained free of disease at all temperatures studied. After 21 days' incubation all inoculated blossoms were rotten and overgrown with sporulating mycelium.

Single spore isolates from these blossoms yielded cultures of the original genera which proved highly pathogenic when healthy Vanda flowers were inoculated with them.

EPIDEMIOLOGY.—Field and laboratory observations indicated a consistent relationship between temperature and infection. This relationship, somewhat elucidated by the preceding experiments, becomes more clearly defined in the following experiment. Each fungus isolate was raised separately on potato-dextrose agar in petri plates at room temperature. When uniform growth was achieved, equally sized fungus-containing agar blocks were removed from each dish and placed in dishes of freshly prepared PDA. Forty dishes of each isolate were prepared and 5 dishes were placed at each of 8 temperatures ranging from 4°-32° C. at 4° intervals. The fungi were incubated for 5 days and the amount of radial growth was measured in mm. The averaged results are represented in Figure 4. *Botrytis* achieved maximal growth at 12° C. and grew at a maximal level through 24° C., above which temperature growth was reduced sharply. Fungi other than *Botrytis* had a more restricted growth temperature range, their maximal growth occurring at or above 20° C. The growth rates of these fungi dropped off at 32° C., with the exception of *Alternaria* strain 51c., which grew well at that temperature.

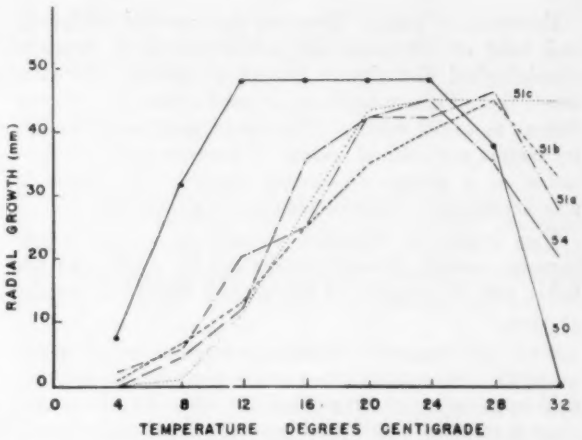

FIG. 4. Temperature and radial growth of five blossom-rotting fungi on potato-dextrose agar in petri dishes. The fungi are *Botrytis* (50), *Gloeosporium* (54), and 3 isolates of *Alternaria* (51a, 51b, and 51c).

Infectivity studies reported previously were apparently conducted within the optimal growth temperature range of *Botrytis*, but somewhat below the temperature optima for the other fungi. This partially accounts for the difference in time of symptom appearance and destructiveness of the various fungi studied. Other unknown fungal characteristics are probably also responsible for these variations.

Average weekly field temperature in a transit rot infested Oahu field was 64° F. for the period November, 1950, through February, 1951. This temperature is optimum for *Botrytis* and approaches the temperature at which *Gloeosporium* grows best. This is below the optima for the strains of *Alternaria*, and no *Alternaria* infection appeared in this field during that period.

The period from November, 1950, through February, 1951, was characterized by cloudy, rainy days and nights and low wind velocities. The resultant high humidity seemed to favor the growth of *Botrytis* and *Gloeosporium* and a high incidence of transit rot. The apparent necessity of free water for germination of these fungi is corroborated by the fact that blossoms which are thoroughly dried before packing seldom suffer from transit rot.

The favorite packing, shipping, and holding temperature is about 55° F. for Vanda blossoms. This fact, in addition to the weather information already related, clearly indicates why transit rot is so prevalent in the winter in certain endemic areas, and why *Botrytis* is usually the principal cause of the disease.

CONTROL. — Vanda blossoms are packed, shipped, and held at relatively low temperatures to avoid a physiological disturbance known as fading. Furthermore, these flowers must be shipped under humid conditions to avoid wilting. The temperature and humidity factors are critical enough to prevent their manipulation as a means of disease control. Therefore, it was necessary to control transit rot chemically.

Two types of chemical control were considered, namely: control through fungicides 1) applied in the field; and 2) applied to the picked flowers as sprays or dips.

Field applications.—Numerous reports indicated the possibility of controlling certain blossom diseases by field application of fungicides (3) (4) (6). However, after 9 months' continuous spraying with many fungicides in a number of locations where flowers showed a high incidence of transit rot, no control of transit rot was obtained. In many cases the fungicides seemed to predispose the blossoms to a greater amount of rotting. The complete failure to control this type of petal blighting by field sprays was in marked contrast to the results obtained in studies of other petal blights (3).

Pack application. — Chemicals to be tested were made up to the desired concentration in water and used as flower dips or sprayed on flowers with a De-Vilbiss hand atomizer. Of 46 chemicals tested, 3 were selected for intensive testing: Natriphene (2-hydroxydiphenyl). Dowicide A (sodium ortho-phenyl-phenate) and dehydroacetic acid.

Visibly disease-free blossoms were picked and laid on a large piece of blotting paper or newspaper. They were then sprayed top and bottom with the test solutions, shaken to remove excess liquid, packed into standard packing boxes, 12 to a box, and sealed. The boxes were stored at 55° F. in the dark for 14 days and observed periodically for transit rot symptoms. No attempt was made to identify the causal fungi by symptoms, but most cases of transit rot seemed to result from *Botrytis* infections. Many flowers were treated in this manner over the 4-month period previously mentioned. Natriphene and Dowicide A were applied at 1/2,000; and dehydroacetic acid was applied at the rate of 1/1,000. The addition of a spreader, Triton B1956, was found to give greater economy of fungicide but no increase in control. Results of these experiments are summarized in Table 2.

Clearly, all 3 materials are highly effective in controlling transit rot, but Natriphene and Dowicide A seem to be less injurious. When DHA was applied at 1/2,000, less injury occurred but percentage control was also reduced.

TABLE 2.—*Results of pack treatment against transit rot of Vanda Orchids*

	Natriphene 1/2,000	Dowicide A 1/2,000	Dehydroacetic Acid 1/1,000	Control
No. of flowers treated	360	120	360	600
No. of flowers diseased	30	10	50	460
No. of flowers injured	10	2	50	0
Percentage diseased	8	8	13	76
Percentage injured	3	2	13	0

SUMMARY

Rotting of Vanda orchid blossoms in shipment was found to be caused by a complex made up of species of *Botrytis*, *Gloeosporium*, and *Alternaria*. The dominance of *Botrytis* in this complex was found to be related to prevalent environmental conditions. Successful control of the rotting was achieved by atomizing the blossoms with various fungicides prior to shipment.

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THE RELATION OF HOST NUTRITION TO THE CONCENTRATION OF CUCUMBER VIRUS 1 IN SPINACH [1]

Pen-Ching Cheo, Glenn S. Pound, and Lewis G. Weathers

Contradictory data have been reported on the relationship between host nutrition and multiplication of certain viruses. Spencer (5) stated that the concentration of tobacco virus 1 in extracts from tobacco plants receiving an ample supply of nitrogen was over 80 times that in extracts from nitrogen-deficient plants. He reported that virus concentration was directly correlated with the nitrogen supplied to the host, even though amounts given were in excess of that required for optimal growth and were causing stunting. Rischkov and Smirnova (4), however, reported that the concentration of tobacco virus 1 in extracts of nitrogen-deficient tomato plants was not lower than in those receiving a normal supply of nitrogen. Recently, Bawden and Kassanis (1) reported that, in general, nutritional conditions most favorable to plant growth are those that increase susceptibility and virus concentration within the host.

This paper reports studies of the effects of host nutrition on the concentration[2] of cucumber virus 1 in spinach (*Spinacia oleracea* L.).

MATERIALS AND METHODS.—Seed of Nobel spinach was sown directly into one gallon glazed earthenware crocks of white silica sand which had been watered with the specific solution under study. Following seedling emergence, all crocks received identical amounts of their specific solutions twice a week. The sand was kept moist but not saturated in order to allow aeration of the root system. Distilled water was added to the crocks, if necessary, between feedings. There were 3 crocks of 10 plants each for each treatment; 2 were inoculated and the third was kept healthy to measure growth.

Modifications of the nutrient solution (1H) formulated by Hoagland and Snyder (3) were used throughout. Chemical composition of the solutions containing different levels of N, P, and K is given in Table 1. All solutions were brought to the same osmotic value by adding sodium chloride where needed. The same amount of minor-element stock solution was added to each of the various solutions. Occasionally, the minor-element solution was atomized onto the foliage in early stages of growth. All solutions were adjusted to pH 6.0.

Virus concentration assays were made with opposite leaf inoculations on cowpea (*Vigna sinensis* Endl. var. Black). Each treatment was compared separately with a standard inoculum from infected tobacco plants. A standard inoculum was prepared for each assay and stored at 4° C. and an aliquot was removed for each comparison. At the end of the series of inoculations of 1 assay (usually 4 to 5 hr.) the stored standard inoculum was inoculated to plants, of which 1 leaf of each had been inoculated at the beginning of the series with an aliquot of the same inoculum. All cowpea plants, except those used as checks for the standard inoculum, were predisposed in darkness for 24 hr. prior to inoculation. Instead of using a standard inoculum as a check in the study of concentration of balanced solutions, each treatment was compared with every other treatment in opposite leaf inoculations to cowpea.

In preparing inoculum for assay, a composite sample of 1 g. of tissue was taken from the youngest leaves of the 20 plants in a treatment and macerated in 5 cc. of a neutral phosphate buffer.

Uninoculated plants for weighing were harvested at the time of the first cowpea assay. At the end of the experiments similar weights were taken of the inoculated plants. Growth of inoculated plants showed the same response to the nutrient concentration as uninoculated plants.

At least 3 virus assays, usually at 5- to 7-day intervals, were made in each experiment.

EXPERIMENTAL RESULTS.—*Nitrogen.*—A series of 4 experiments was carried out from July through November, 1950. The first had 5 levels of nitrogen (21, 210, 420, 630, and 1050 p.p.m.). Plants at the 21 p.p.m. level were so deficient that they made very little growth, were very yellow, and bolted to seed very early. There was throughout the experiments a general correlation between the nitrogen level and the earliness of bolting, the lower the nitrogen the earlier the production of seed stalks. The virus concentration in plants at the 21 p.p.m. level was also very low in all assays. Plants grown at 210 and 420 p.p.m. showed little difference in vegetative growth. In subsequent trials, the 21 and 420 p.p.m. solutions were omitted and the 5 levels used were 70, 210, 630, 1050, and 1330 p.p.m. In each of the 3 trials with these levels, plants grown at 630 p.p.m. produced the most growth, as measured by wet and dry weights. Above that level, plants showed noticeable stunting but were much darker green. Results of the virus assays (Table 2) showed that the virus concentration coincided directly with the amount of vegetative growth produced. Concentration increased up to 630 p.p.m., beyond which it declined. A sharp drop of virus concentration at 1050 and 1330 p.p.m. coincided with a corresponding retardation of growth. This was true in all assays. Symptoms appeared at the same time in all groups of plants and, apparently, the virus concentration at each nitrogen level reached its peak at about the same time, for the interval assays of any 1 experiment showed the same picture. This was contrary to Spencer's (5)

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[2] The term virus concentration in this paper refers to the virus infectivity of expressed plant saps as measured by local lesion counts.

TABLE 1.—*Chemical composition of nutrient solutions to obtain different levels of nitrogen, phosphorus, and potassium*

Salt	Molarity of stock solution	Amount (cc) of stock solution per liter of final solution to get concentration (p.p.m.) of																	
		Nitrogen							Phosphorus						Potassium				
		21	70	210	420	630	1050	1330	3	10	31	93	237	547	8	78	430	704	1408
$Ca(NO_3)_2.4H_2O$	2.5			4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
KNO_3	2.0	0.75	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5			2.5	2.5	2.5
$NaNO_3$	6.0				2.5	5.0									0.83	0.83			
NH_4NO_3	6.0						5.0	6.67											
KCl	3.0	1.16							0.30	0.22					0.07	0.67	2.0	4.0	10.0
KH_2PO_4	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.1	0.33	1.0	1.0	1.0	1.0				1.0	1.0
$MgSO_4.7H_2O$	2.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
$CaCl_2.2H_2O$	4.0	1.25	1.25																
$NaH_2PO_4.H_2O$	2.0											1.0	3.33	8.33	0.50	0.50	0.50		
NaCl	5.0	8.0	8.50	8.0	4.44	2.0	2.0		6.09	6.09	5.99	5.40	4.0	1.0	5.96	5.59	4.79	3.59	

report with tobacco virus 1 in which he pointed out that the maximum virus concentration was reached earlier with a low or medium supply of nitrogen than with an excess of nitrogen.

The relationship between virus concentration and growth at the different nitrogen levels is shown in Figure 1. The graph showing virus concentration was made by taking the overall average of the checks in all comparisons and then computing the number of local lesions for each treatment by using its relationship to the respective check.

Phosphorus.—Three experiments were carried out during the period, December through March. The first had the following 5 levels of phosphorus (3, 10, 31, 93, and 237 p.p.m.). The difference of growth of plants at different levels was not so marked as in the nitrogen experiments. Growth response seemed to vary with the season. In a trial made during the summer, plants grown at 3 p.p.m. made little growth, more or less equivalent to the 21 p.p.m. nitrogen solution. During the winter, however, plants at 3 p.p.m. of phosphorus made fairly good growth, although visibly less than that at 10 p.p.m. In all trials there was a gradual increase in growth up to 93 p.p.m., after which it fell off slightly (Table 3). Little difference in color existed between the different levels, as in the nitrogen experiments. With phosphorus, too, time of bolting was directly related to the amount of the element supplied; the lower the phosphorus, the earlier the bolting.

The results of the virus assays in the first trial showed that the concentration was highest in plants grown at 237 p.p.m., at which level growth was less than at 93 p.p.m.

TABLE 2.—*Effect of different amounts of nitrogen on host growth and on concentration of cucumber virus 1 in spinach*

Opposite leaf comparisons	Ave. number of local lesions per cowpea leaf and ave. weight per spinach plant in trial and assay interval indicated					
	Trial 1-8 days			Trial 2-10 days		
	Lesions	Fresh weight	Dry weight	Lesions	Fresh weight	Dry weight
		(gm.)	(gm.)		(gm.)	(gm.)
Check [a]	176.4					
21 p.p.m.	0.0	0.49	0.05			
Check				316.8		
70 p.p.m.				19.5	0.91	0.06
Check	151.4			326.5		
210 p.p.m.	192.4	2.11	0.15	40.1	1.15	0.07
Check	148.9					
420 p.p.m.	155.5	2.12	0.16			
Check	145.2			365.4		
630 p.p.m.	302.4	2.63	0.19	50.6	1.70	0.11
Check	109.3			248.9		
1050 p.p.m.	24.7	2.28	0.18	13.9	0.77	0.06
Check				291.3		
1330 p.p.m.				12.9	0.63	0.05

[a] Check refers to a standard inoculum of cucumber virus 1, an aliquot of which was used for each comparison.

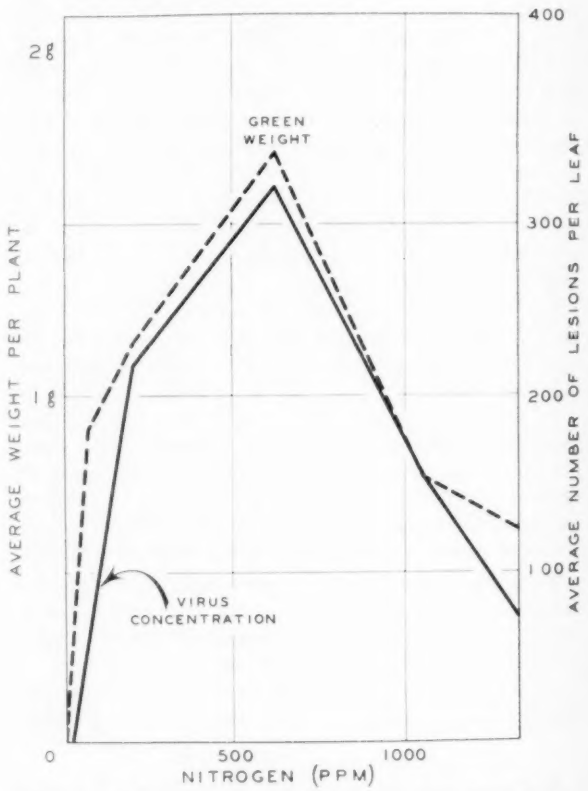

FIG. 1. The effect of nitrogen on host growth and concentration of cucumber virus 1 in Nobel spinach as expressed by the number of local lesions on cowpea.

TABLE 3.—*Representative data showing the effect of different amounts of phosphorus on host growth and on concentration of cucumber virus 1 in spinach (Trial 3)*

Opposite leaf comparisons	Average number local lesions at intervals after inoculation		Average weight of healthy plants [b]	
	9 days	13 days	Fresh	Dry
Check [a]	34.0	31.2		
3 p.p.m.	0.4	17.1	1.94 gm.	0.23 gm.
Check	36.7	24.2		
10 p.p.m.	114.2	214.5	4.45 gm.	0.39 gm.
Check	40.3	22.1		
93 p.p.m.	218.3	272.4	4.60 gm.	0.35 gm.
Check	33.5	21.6		
237 p.p.m.	245.2	299.1	3.86 gm.	0.32 gm.
Check	47.5	20.2		
547 p.p.m.	262.5	304.2	3.16 gm.	0.32 gm.

[a] Check refers to a standard inoculum taken from tobacco, an aliquot of which was used for each comparison.
[b] At 9 days after inoculation.

TABLE 4.—*Effect of different amounts of potassium on host growth and on concentration of cucumber virus 1 in spinach*

Opposite leaf comparison	Number of local lesions and weight of plants in trial and assay interval shown					
	Trial 1-15 days			Trial 2-15 days		
	Lesions	Fresh weight	Dry weight	Lesions	Fresh weight	Dry weight
		(gm.)	(gm.)		(gm.)	(gm.)
Check [a]	48.0			118.0		
8 p.p.m.	39.2	1.72	0.15	52.1	2.98	0.24
Check	30.3			131.4		
78 p.p.m.	100.6	3.78	0.29	138.1	4.62	0.35
Check	36.1			91.3		
430 p.p.m.	112.8	4.37	0.36	290.3	6.49	0.47
Check	43.9			101.3		
704 p.p.m.	100.9	4.07	0.32	177.7	3.99	0.31
Check	34.1			91.9		
1408 p.p.m.	65.8	3.65	0.29	178.9	4.10	0.32

[a] Check refers to a standard inoculum of cucumber virus 1, an aliquot of which was used for each comparison.

In order to obtain a further retarding effect of excessive phosphorus on growth of the plants and to see if the increase of virus concentration with increased phosphorus was directly related to the plant growth or to the amount of phosphorus supplied, a second trial was made with the following levels: 3, 10, 93, 237, and 547 p.p.m. This experiment was carried out during February and March in reduced day lengths and light intensity, and the growth differences manifested by the first trial were absent here. Even at 547 p.p.m. stunting was not marked. Plants at this level had the highest average dry weight, although the highest fresh weight was from the 93 p.p.m. level. Virus assays showed little difference in concentration between the 93, 237, and 547 p.p.m. levels.

A third experiment was performed the following May when day length was again longer and when light intensity was higher than in the second trial. In this trial, as in the first, a gradient of growth occurred, with the maximum fresh weight of plants occurring at the 93 p.p.m. level but with the maximum dry weight at 10 p.p.m. level. Marked stunting occurred at the 2 highest levels. Virus assays, however, showed a concentration gradient with the lowest concentration at the lowest phosphorus level and the highest concentration at the highest phosphorus level (Table 3). However, the differences between the 93, 237, 547 p.p.m. levels were slight. It is significant that the virus concentration did not drop with retardation of growth.

Potassium.—The 5 levels of potassium used were 8, 78, 430, 704, and 1408 p.p.m. In 1 experiment only slightly reduced growth was noticed on plants grown at 8 p.p.m., while plants at the other levels showed almost identical growth. The highest fresh and dry weights per plant were obtained at the 430 p.p.m. level. In a second experiment the greatest growth was again obtained at 430 p.p.m. with pronounced stunting above and below this level (Table 4). In both trials, plants grown at 1408 p.p.m. tended to bolt somewhat earlier than plants grown at 8 p.p.m., a reverse situation to nitrogen and phosphorus.

In the virus assays, the concentration curve in both trials tended to coincide closely with the growth curve (Table 4). The highest concentration was indicated in plants at 430 p.p.m. where the greatest growth occurred and the lowest concentration was from plants showing the least growth.

Concentration of Balanced Solutions.—Plants were grown in a balanced solution in which the concentration of nutrients was the variable. The solution was that described by Hoagland and Snyder (3). With the basic solution being represented by 1H, the following concentrations were used: 0.5H, 1H, 2H, and 3H. Thus the concentration of the 3H solution was 6 times that of the 0.5H solution. In the 1H level the concentrations of N, P, K were 210, 31, and 235 p.p.m., respectively. Two experiments were performed in late summer of 1951 (Table 5) and in each the greatest growth was at the 1H level with marked stunting occurring at the higher levels. In these virus assays, instead of using a standard inoculum to compare with each treatment, each nutrient level was compared with each of the other levels in opposite leaf comparisons. Each group was analyzed separately. The differences in all of the comparisons were highly significant except in the second experiment in which no difference occurred between the 0.5H and 2H levels. Three separate assays were run for each experiment and in each assay the same picture occurred as shown in Table 5. Lesion counts in Table 5 are the overall averages for each inoculum and are presented thus for simplification.

It can be seen that the virus concentration agreed closely with host growth.

DISCUSSION.—In these studies, using the local lesion reaction of cucumber virus 1 on cowpea, the measurements obtained were of virus infectivity in plant ex-

TABLE 5.—*Effect of concentration of balanced solution on host growth and concentration of cucumber virus 1 in spinach*

Nutrient concentration	Average number local lesions and average plant weight in trial and assay interval shown: Trial 1 Lesions	Trial 1 Fresh weight	Trial 1 Dry weight	Trial 2 Lesions	Trial 2 Fresh weight	Trial 2 Dry weight
		(gm.)	(gm.)		(gm.)	(gm.)
0.5H	67.0	2.08	0.15	225.4	2.17	0.21
1.0H	92.9	2.59	0.19	373.3	2.83	0.28
2.0H	47.2	1.34	0.14	220.1	1.84	0.18
3.0H	15.8	0.98	0.10	161.1	1.22	0.12

tracts rather than of total virus concentration. The relative infectivity, however, should be directly correlated with total concentration unless certain factors such as pH, presence of inhibitors, etc., would vary between the assay preparations. Spencer (7) in his study of the effect of nitrogen nutrition on the concentration of tobacco virus 1 in tobacco suggested the possibility that the nutrition may have been correlated with the amount of inactivators in the plant tissue. This would imply a differential rate of inactivation rather than a differential rate of virus production. Spencer (6, 8) also suggested that nitrogen increased infectivity even when the amounts given were in excess of those needed for optimal growth and were causing plant stunting. He reported in 1941 (6) that the total virus content of nitrogen-deficient leaves remained constant for 16 days while the infectivity fell by 50 per cent, whereas in leaves receiving adequate nitrogen the virus increased fivefold and was not reduced at all in infectivity. Chessin (2) has also shown a reduced infectivity of tobacco virus 1 when taken from nitrogen deficient tobacco plants with Golden Cluster bean as the assay host. No reduced infectivity was evident, however, if *Nicotiana glutinosa* was the assay host.

Bawden and Kassanis (1) were unable to demonstrate any consistent differences in infectivity of tobacco mosaic virus obtained from plants supplied with widely different quantities of nitrogen, phosphorus, and potassium. .

In these studies, the results in general agreed with conclusions of Bawden and Kassanis (1) in that nutritional conditions most favorable to plant growth were also those that favored virus multiplication. However, in the phosphorus experiment, the results showed an increase in concentration with increased phosphorus, even though growth was retarded at the highest level. The response of spinach to high phosphorus, however, was less marked than the response to high nitrogen. Also, the amount of phosphorus required for optimal growth varied considerably according to the season of year. In a trial in December, the best growth both in fresh and dry weight occurred at 93 p.p.m., but in a trial in March the highest fresh weight was from 93 p.p.m. while the highest dry weight was from 547 p.p.m. In still another trial in January the highest fresh and dry weight was from plants at 547 p.p.m. Thus seasonal conditions apparently influence the phosphorus requirement of spinach. It is evident, however, that a much higher phosphorus level is required to retard virus multiplication than to reduce plant growth. Bawden and Kassanis (1), working with tobacco virus 1, reported that supplements of phosphorus produced the greatest increases in plant size, in virus concentration of expressed sap and in total virus content per plant, and that nitrogen increased plant size only when phosphorus was added and only then increased virus concentration and total virus per plant. Combined supplements of phosphorus and nitrogen doubled the virus concentration of sap and increased the total virus per plant by factors up to 40.

In studying the role of potassium, Bawden and Kassanis (1) mentioned that increase in potassium slightly reduced the virus concentration of sap, although it usually increased plant size and total virus per plant. In the present study it was found that the amount of potassium that increases growth of plants also increases virus multiplication and vice versa.

SUMMARY

The role of nitrogen, phosphorus, potassium, and balanced solution concentration in relation to plant growth and the concentration of cucumber virus 1 in spinach has been studied. In the case of nitrogen, spinach plants showed an optimum growth at 630 p.p.m. Above that level, marked stunting occurred; virus assays showed that the virus concentration in expressed sap coincided closely with the growth of the plants. The level of nitrogen that increased growth at the same time increased virus concentration, and vice versa.

With phosphorus, the growth response seemed to vary with the season. In general, a growth gradient occurred with maximum growth occurring at 93 p.p.m. Some stunting occurred at higher levels (547 p.p.m.) although this was much less marked than with nitrogen. Virus assays indicated that phosphorus levels high enough to effect stunting did not similarly retard virus multiplication but, on the contrary, increased it. The highest virus concentration occurred at the highest phosphorus level.

In the cases of potassium and the concentration of balanced solutions, virus concentration appeared to be directly related to growth. Concentration levels yielding the greatest growth also yielded the greatest virus concentration.

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INDEPENDENT TRANSLOCATION OF SAP-TRANSMISSIBLE VIRUSES[1]

Daniel A. Roberts

The literature on movement of plant viruses within suscepts has been reviewed in detail (1, 13, 15, 16, 33). There is much evidence that long distance movement, or translocation, of plant viruses is correlated with translocation of elaborated food materials. However, there are a few reports which indicate that, under certain conditions, virus translocation may not be connected with that of sugars. It has been demonstrated that in potato plants infected by virus X, virus concentration appears to be low in young tubers (1, 28) despite the rapid inward flow of large quantities of carbohydrates. Caldwell (10) found that virus moved systemically out of darkened inoculated leaves, presumably against a sugar gradient. Curtis and Clark (18) point out that while most viruses appear to move along with food materials, their movement could be independent. Some of the same agents which normally regulate and influence translocation also could affect virus movement.

Experiments were designed to test the hypothesis that translocation of sap-transmissible viruses in plants can occur independently and may not necessarily be correlated with the translocation of food materials.

MATERIALS AND METHODS. — Isolates of tobacco-mosaic virus (*Marmor tabaci* H.), cucumber-mosaic virus (*M. cucumeris* H.), tobacco-ringspot virus (*Annulus tabaci* H.), and potato virus X (*A. dubius* H.) were used. Suscepts were either vegetative Turkish tobacco (*Nicotiana tabacum* L.) plants or Scotia bean (*Phaseolus vulgaris* L.) plants with 1 to 3 trifoliate leaves. Inocula were prepared from infected Turkish tobacco leaves. Leaves were ground with mortar and pestle, crude juice expressed through cheesecloth and diluted with measured amounts of distilled water. Leaves dusted with carborundum were inoculated by means of cheesecloth pads saturated with inoculum. Inoculated leaves were rinsed with tap water within 5 min. after inoculation.

Light was excluded from some leaves after inoculation by 12 x 18 cm. aluminum-painted black paper envelopes fastened with paper clips. In certain tests, portions of the stem or petiole below inoculated leaves were killed by scalding to prevent flow of sugars past inoculated leaves (Fig. 1). In these tests, a 1-cm. band of absorbent cotton was wrapped around the stem or petiole, and then saturated with about 5 ml. of boiling water. Illumination was provided by a 500-watt Mazda lamp in a porcelain reflector suspended 1 m. above the greenhouse bench.

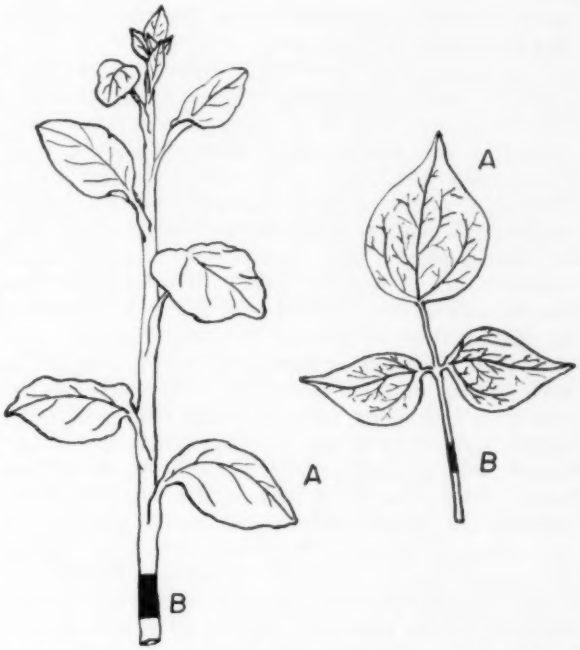

FIG. 1. Tobacco shoot (left) and Scotia bean leaf (right). A, leaf or leaflet selected for inoculation. B, region of stem or petiole selected for scalding.

Virus was recovered from various parts of inoculated plants by inoculations to appropriate indicator plants. Tobacco-mosaic virus, cucumber-mosaic virus, and potato virus X were recovered in the local-lesion suscepts *Nicotiana glutinosa* L., *Chenopodium hybridum* L. (26), and *Gomphrena globosa* L. (32), re-

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spectively. Tobacco-ringspot virus was recovered in Turkish tobacco.

The starch-iodide reaction was used to determine the presence of starch in every experiment where leaves were darkened. Leaves were killed in boiling water, and cleared in 2 successive baths of boiling 95 per cent alcohol. Cleared leaves were rinsed in distilled water, spread on glass plates, and flooded with a weak solution of iodine in potassium iodide.

TISSUE RELATIONS.—With the exception of the virus of Pierce's disease of grape (*Morsus suffodiens* H.), translocation of virus appears to occur predominantly in the phloem (1, 6, 9, 16). Evidence to the contrary, such as the movement of tobacco-mosaic virus across steamed areas of tobacco stems (21), can be explained on the assumption that translocation occurred through intact internal phloem underlying the regions which had been killed by steaming.

The Scotia bean, which lacks internal phloem and which becomes systemically infected by tobacco-ringspot virus, was used in a study of the tissues concerned in virus translocation. Terminal leaflets of 20 plants were inoculated with tobacco-ringspot virus. Petioles of 17 plants were scalded below the lateral leaflets, and 3 plants were kept as unscalded controls. In treated plants, necrosis was restricted to lateral leaflets distal to scalded areas. All controls became systemically infected as evidenced by the development of characteristic symptoms. Necrotic ringspots and veinstreak developed in inoculated leaves. During the 4-wk. course of the experiment, virus could not be recovered from plant parts separated from inoculated leaflets by scalded areas. Tobacco-mosaic, cucumber-mosaic, tobacco-ringspot, and potato X viruses did not pass scalded areas of 45 tobacco plants used in other experiments. These results are consistent with most of the available evidence, which indicates that the phloem is the chief tissue concerned in virus translocation.

INDEPENDENT TRANSLOCATION OF VIRUSES AND CARBOHYDRATES.—The hypothesis that virus translocation is correlated with that of food materials is supported by reports of rapid virus movement into defoliated or darkened shoots (4, 5, 7, 8, 12, 22) and developing fruit (29), and slow virus movement out of etiolated leaves (7) or infected roots (8, 19, 25, 27). However, Caldwell (10) has presented evidence that, under certain conditions, the aucuba strain of tobacco-mosaic virus can be translocated in directions opposite to those in which food materials are moving in tobacco or tomato plants. When 1 leaf of starch-free plants was inoculated, systemic spread of virus was unaffected by position of the rubbed leaf and by darkening or defoliation. The validity of the assumption that sugars moved into darkened inoculated leaves as virus moved systemically was not tested, and there remained the possibility that diurnal reversals of flow of materials in the phloem might have accounted for the results.

Bennet (7) found that dark treatments had little or no effect upon movement of sugar-beet curly-top virus (*Ruga verrucosans* Carsner and Bennett) in Turkish tobacco shoots. Plants about 2 ft. high were pruned to a height of about 6 in., and shoots from the upper 2 buds were allowed to grow. Darkening inoculated or noninoculated shoots before or after inoculation did not influence virus movement. The objection was raised that dark treatments make so uncertain the direction of food movement in tobacco shoots that any correlation between virus and food translocation would be difficult to detect, and that the failure of darkening to affect virus movement would not be proof that it is independent of food translocation (7).

TABLE 1.—*Systemic infection of tobacco plants kept in light after inoculation of 1 starch-free leaf*

Virus	No. plants	Treatment of rubbed leaf	Period of latent infection[a]
			(days)
TMV	8	Dark	10
	6	Light	7
TRSV	8	Dark	10
	4	Light	8
PVX	2	Dark	11
	1	Light	7

[a] Average time elapsed between inoculation and appearance of systemic symptoms.

Tests were designed to eliminate diurnal reversals of flow of materials in the phloem and the uncertainty as to movement of sugars into darkened inoculated leaves. In 7 experiments, a total of 45 Turkish tobacco and 24 Scotia bean plants were darkened for 3 days, a period which proved sufficient to deplete them of starch. One lower leaf of each tobacco plant was inoculated with tobacco-mosaic virus (TMV), potato virus X (PVX), or tobacco-ringspot virus (TRSV), and 1 terminal leaflet of each Scotia bean plant was inoculated with tobacco-ringspot virus. Each stem of 16 tobacco plants was scalded below the inoculated leaf, and all Scotia bean petioles were scalded below lateral leaflets. Some inoculated leaves were darkened, and the plants kept in continuous light.

Although all plants became systemically infected in all parts above scalded sections, symptoms were delayed in plants where inoculated leaves had been darkened (Tables 1 and 2). Starch accumulated along

TABLE 2.—*Systemic infections and starch accumulation in rubbed leaves or leaflets above scalded stems or petioles. Plants were depleted of starch before inoculation, and kept in light after inoculation*

Virus	Suscept	Treatment of rubbed leaf	Starch accumulation[a]	Period of latent infection[b]
				(Days)
TMV	Tobacco	Dark	8/10	10
		Light	6/6	7
TRSV	Scotia Bean	Dark	16/16	14
		Light	8/8	10

[a] Numerator is the number of leaves showing positive starch test, and the denominator the total number of inoculated leaves tested.

[b] Average time elapsed between inoculation and appearance of systemic symptoms.

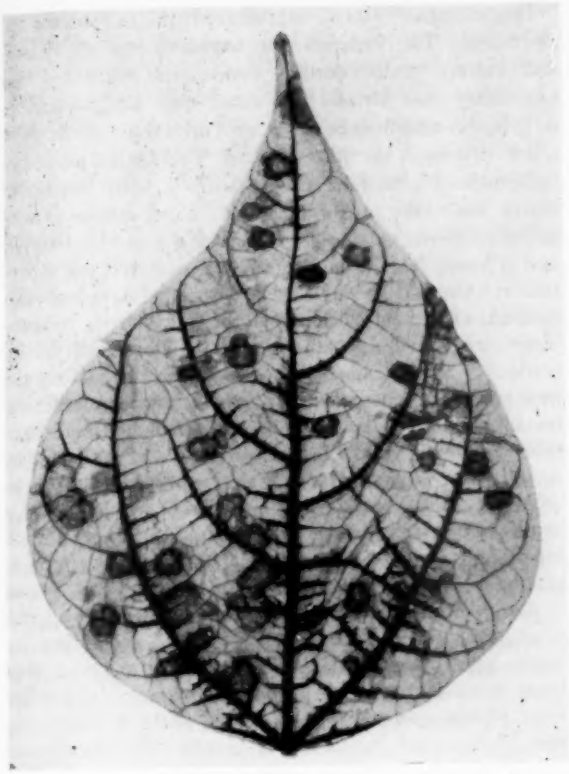

FIG. 2. Iodine-treated Scotia bean leaflet which was starch-free when inoculated with tobacco-ringspot virus, and subsequently kept darkened for 8 days. The plant was illuminated continuously after inoculation. Petiole had been scalded below the lateral leaflets. (Photograph by H. H. Lyon, Jr.)

midribs and veins of darkened leaves (Table 2; Fig. 2). Stems below the 2 tobacco leaves in which starch did not accumulate were not scalded as completely as others. It is likely that translocation to roots was not blocked sufficiently to divert excess sugar into darkened inoculated leaves. Virus was recovered from growing tips of all tobacco plants 4 days after inoculation, and from all Scotia bean leaflets showing symptoms. While these tests were not quantitative, inoculum from control tobacco plants consistently caused more local lesions than did that from treated plants. Under the conditions described, translocation of virus in the phloem occurred in a direction opposite to that in which sugars were moving. That sugars moved against the flow of virus is evident from the fact that starch accumulated in the darkened leaves while virus moved out of them. During this time, the plants were under continuous illumination, thus eliminating the possibility of diurnal changes in the direction of flow of sugars. These results provide evidence for a translocation mechanism enabling bi-directional movement of materials in the phloem.

The delay in systemic symptom development in plants where inoculated leaves were darkened might be interpreted as the result of a decreased rate of virus translocation caused by flow of sugars in an opposite direction. Actually, virus was recovered from the tips of treated plants as soon as from those of controls, but virus titer, as indicated by local lesion counts, was higher in the controls. Dark treatments could have influenced the quantity of virus translocated rather than the velocity of systemic spread. The possibility that darkening decreased virus multiplication in inoculated leaves was tested, as described in the following section.

VIRUS MULTIPLICATION IN INOCULATED TOBACCO LEAVES.—Pre-inoculation darkening or shading seemed to enhance establishment of primary infections by certain plant viruses in local-lesion suscepts (2, 3, 23, 35), but dark treatments had an opposite effect when *Gomphrena globosa* leaves were rubbed with potato virus X (31) and when healthy and mosaic leaves of *Nicotiana sylvestris* Speg. & Comes were inoculated with a local-lesion strain of tobacco-mosaic virus (20). The influence of darkening upon virus multiplication rates in inoculated leaves where there is no local necrosis apparently has not been reported.

Twelve Turkish tobacco plants were kept in complete darkness for 3 days, after which a lower leaf of each was rubbed with a 1:100 dilution of juice of tobacco plants infected by potato virus X. Half the inoculated leaves were darkened, and all plants were kept under continuous illumination. At 2-day intervals, 2 inoculated leaves were harvested from each group of plants, and relative virus titer was assayed. Crude juice was diluted 1:100 with water, and 16 pairs of *Gomphrena globosa* leaves were inoculated. One leaf of each pair was rubbed with juice from the controls, and the other with juice from the darkened leaves. The inoculation scheme provided for randomization with respect to leaf position, order of inoculation, and time of rinsing.

Inoculum from illuminated leaves invariably caused the formation of more local lesions than did that from darkened leaves (Table 3). Since all tobacco plants had received the same pre-inoculation treatment, the influence of subsequent darkening must be attributed to its effect upon virus multiplication rather than upon establishment of infection. Virus was recovered from growing tips of all plants 4 days after inoculation, but not as early as 2 days after inoculation. Recovery was not attempted on the third day after inoculation.

TABLE 3.—*Local lesions in Gomphrena globosa leaves rubbed with similarly diluted juice from darkened and illuminated inoculated tobacco leaves*

Days after inoculation of tobacco leaves	Tobacco leaves in [a]	
	Dark	Light
2	2	5
4	89	112**
6	317	487**

[a] Each figure in these colums is the total for 16 rubbed leaves.

** Denote mathematical significance at the 1 per cent level between counts for light and dark samples.

TABLE 4.—*Average number of local lesions per half-leaf of Nicotiana glutinosa inoculated with tobacco-mosaic virus from frozen and thawed tobacco leaves harvested 4 days after inoculation*

Pre-inoculation treatment of tobacco plants	Treatment of inoculated tobacco leaves	Local lesions [a]
Greenhouse	Dark	134
	Light	148
Dark	Dark	117
	Light	138
L. S. D., Odds 19:1		16
L. S. D., Odds 99:1		21

[a] Each of the first 4 figures in this column is the average for 36 half-leaves on 12 plants. Inoculated leaves were arranged according to a randomized incomplete block design.

The rate of multiplication of tobacco-mosaic virus in inoculated tobacco leaves was determined. Sixteen tobacco plants were divided into 2 equal groups, 1 of which was kept in the dark for 3 days while the other was kept in the greenhouse. One lower leaf of each plant was rubbed with a 1:10 dilution of juice of tobacco plants infected by tobacco-mosaic virus. Six of the inoculated leaves in each group were darkened. All stems were scalded below inoculated leaves, and the plants were illuminated continuously. Inoculated leaves were harvested and frozen 4 days following inoculation. Inocula were prepared from the frozen and thawed material 3 wk. later, and consisted of 4 solutions prepared at a dilution of 1:100. Two solutions, 1 from leaves darkened and the other from leaves illuminated after inoculation, were prepared from each group of plants. Each was rubbed on 36 half-leaves of *Nicotiana glutinosa* in a randomized incomplete block design (34) involving 12 plants.

Evidence was obtained for higher virus titer in tobacco leaves illuminated after inoculation than in those which had been darkened, particularly if leaves were starch-free at the time of inoculation (Table 4). There was somewhat less virus after 4 days in rubbed leaves which had been darkened before inoculation than in those kept in the greenhouse. Where leaves were illuminated after inoculation, the influence of pre-inoculation darkening was not mathematically significant.

In these experiments, dark treatments of inoculated leaves seemed to decrease virus multiplication rates. This could account, at least in part, for the increased period of latent infection when rubbed leaves were darkened. As a result of the reduced multiplication rate in leaf parenchyma, less virus would reach the phloem during a given time interval. Consequently, in a given time, less virus would be translocated to receiving tissues. With sap-transmissible viruses, the rate of development of systemic symptoms seems to be a function of the number of particles introduced into receiving tissues. Therefore, it seems reasonable that systemic symptom development in the experiments reported in this paper was limited by the amounts of virus translocated from inoculated leaves.

INDEPENDENT VIRUS TRANSLOCATION IN MIXED INFECTIONS.—The independent translocation of viruses and sugars under certain conditions suggested the possibility that viruses can move over long distances in plants, uninfluenced by the direction of flow of other viruses in the phloem. Six Turkish tobacco and 6 *Nicotiana glutinosa* plants with 5 to 7 expanded leaves were kept in the dark for 3 days, a time proved to be sufficient to deplete them of starch. An upper and a lower leaf of each plant was selected for inoculation. One such leaf of each starch-free plant was rubbed with a 1:100 dilution of juice from tobacco plants infected by potato virus X (PVX). Half the inoculations were made on a lower leaf and half on the first expanded leaf below the growing tip. The other selected leaf of each tobacco plant was inoculated with tobacco-mosaic virus (TMV), while that of each *N. glutinosa* plant was inoculated with cucumber-mosaic virus (CMV). Both inocula had been prepared by diluting crude infectious tobacco juice 1:100 with water. Both inoculated leaves of 2 tobacco and 4 *N. glutinosa* plants were darkened, while both inoculated leaves of 2 plants of each species were left exposed to light. The lower inoculated leaves of the 2 remaining tobacco plants were darkened. One of the leaves had been inoculated with tobacco-mosaic virus, the other with potato virus X. Stems were scalded below the basal inoculated leaf, and the plants were illuminated continuously.

Both tobacco-mosaic and potato X viruses were recovered from growing tips of all tobacco plants 7 days after inoculation. Both viruses were recovered from all of the inoculated tobacco leaves which were tested 10 days after inoculation (Table 5). Inocu-

TABLE 5.—*Recovery of viruses from starch-free inoculated leaves of Nicotiana tabacum and N. glutinosa*

Suscept	Virus used to inoculate: Lower leaf [a]	Virus used to inoculate: Upper leaf [a]	Virus recovered from: Lower leaf	Virus recovered from: Upper leaf	Period of latent infection [b]
					(days)
N. tabacum	(PVX)	(TMV)	Both [c]	Both [c]	10
	(PVX)	TMV	Both [d]	TMV [d]	9
	PVX	TMV	Both [c]	Both [c]	8
	(TMV)	(PVX)	Do	Do	9
	(TMV)	PVX	Do	Do	9
	TMV	PVX	Do	Do	7
N. glutinosa	(PVX)	(CMV)	Both [e]	Both [e]	15
	(PVX)	(CMV)	Do	Do	16
	PVX	CMV	Do	Do	12
	(CMV)	(PVX)	Do	Do	14
	(CMV)	(PVX)	Do	Do	16
	CMV	PVX	Do	Do	13

[a] Use of parentheses indicates that leaf was kept in the dark after inoculation. All leaves were starch-free at time of inoculation.

[b] Time elapsed between inoculation and appearance of systemic symptoms.

[c] Viruses recovered 10 days after inoculation.

[d] Viruses recovered 4 days after inoculation.

[e] Viruses recovered 19 days after inoculation.

lated leaves were harvested from 1 plant 4 days after inoculation. The upper leaf had been rubbed with tobacco-mosaic virus and illuminated, and the lower one had been rubbed with potato virus X and darkened. Both viruses were recovered from the lower leaf, but only tobacco-mosaic virus was recovered from the upper one. Virus X had moved out of the inoculated leaf by this time, since it was recovered from growing tips later, but no detectable infection of the upper inoculated leaf had occurred.

Both potato virus X and cucumber-mosaic virus were recovered from each inoculated *N. glutinosa* leaf 19 days after inoculation. Systemic symptoms were delayed in plants where inoculated leaves had been darkened, presumably as a result of decreased virus multiplication in and less virus translocation out of darkened leaves.

Dark treatments which would be expected to alter the direction of flow of food materials did not appear to influence systemic spread of viruses in mixed infections, and the translocation of 1 virus was not measurably influenced by that of another. Continuous illumination of all plants after inoculation resulted in establishment of sugar gradients into darkened leaves. While sugars moved into darkened leaves, as evidenced by positive starch tests, at least 1 virus was moving in the opposite direction. Furthermore, the movement of 1 virus was in a direction opposite to that of another virus in each plant, regardless of dark treatments.

Discussion.—Under proper environmental conditions, some sap-transmissible viruses can move long distances in the phloem against the direction of flow of sugars. Translocation of 1 virus seems not to be influenced by the direction of movement of another virus in the same plant. This supports the hypothesis of independent translocation of viruses and other phloem-translocated materials. It is probable that the same factors influence translocation of all materials in the phloem (8, 18), and that a given material moves independently of others. The results of experiments described here can be explained only on the assumption that the translocation mechanism in plants can provide for bi-directional flow of materials in the phloem.

While there is no doubt as to the direction of virus and sugar movement in the experiments reported here, there might be some question concerning the movement of other materials, particularly the breakdown products of protein metabolism in darkened leaves. It could be argued that virus movement is correlated with that of amino acids or other materials, and that this might account for virus translocation out of darkened leaves agaisnt sugar gradients. Even if this were true, evidence for independent translocation of viruses and sugars would not be invalidated. It seems unlikely that much material of any kind was moving out of leaves after they had been in the dark for as long as 3 days. Schumacher (30) reported that there was active emptying of carbohydrates from darkened leaves for only 2 to 3 hours, after which there was a rapid removal of nitrogen for another 24 hours. Neither carbohydrate nor nitrogen was removed after 3 days in the dark. There is no reason to suspect that appreciable amounts of food materials moved out of starch-free Turkish tobacco leaves or Scotia bean leaflets after they had been inoculated. There was certainly a net gain of carbohydrate in the darkened leaves.

Bennett (7) presented evidence that viruses are translocated in the phloem along with elaborated food materials. When sugar-beets were split longitudinally into 3 parts connected at the base, leaf shoots developed from each of the 3 crowns. When 1 shoot was inoculated with curly-top virus, symptoms appeared within 2 wks. on another shoot which had been darkened or defoliated. Untreated shoots remained healthy or did not become infected until several months after inoculation. If shoots which had remained virus-free were defoliated and placed in the dark for 5 days, they became diseased within 12 days. Holmes (22) and Cochran (12) also reported that darkening or defoliation seemed to hasten movement of virus into treated regions.

Bawden (1) points out that darkening and defoliation may create conditions which favor establishment of infection and multiplication of virus in receiving tissues rather than affecting the rates at which viruses move. In some cases, darkening and shading appear to increase the likelihood of virus becoming established in primary infections (2, 3, 23). Treatments might so influence those factors which control both virus and sugar translocation that either or both would move rapidly into treated parts, provided both were available in other parts of the plants. It is unnecessary to postulate that different factors control translocation of sugars and viruses. In leaves such as those used in the present studies, even though movement of virus and sugar might have been under the control of the same factors, the absence of sugar in leaves at the time of inoculation would not have had any major effect upon movement of virus out of such leaves. It would be useless to speculate further than to suggest that the effects of dark treatments and defoliation upon respiration, cyclosis, and, possibly, auxin concentration might be such that they could account for the results obtained.

Bawden (1) suggests that the preferential movement of virus into developing fruit (29) and the slow movement out of etiolated leaves (7) is the strongest evidence that virus and sugar translocation are correlated. This view is supported by reports that virus moves slowly, if at all, from inoculated roots to the tops of plants (8, 19, 25, 27). The apparent slow movement out of etiolated leaves and inoculated roots might be a consequence of a decreased volume of virus being translocated because of decreased multiplication rates in inoculated tissues, the amounts translocated being insufficient to establish infection in receiving tissues. The rapid movement of virus into developing fruit is

not necessarily caused by the rapid inward flow of sugars. Other factors could be responsible for mobilization of both viruses and sugars, each of which might actually move independently.

The translocation of viruses in plants has been explained on the assumption that a mass flow mechanism (14) provides for translocation of materials in the phloem (7, 8). However, in the experiments reported here, translocation of each of 4 sap-transmissible viruses occurred independently, and was not measurably influenced by the direction of flow of sugars or of another virus. These results are in close agreement with those obtained where fluorescein (24, 30) and radioactive tracer elements (11) were used in translocation studies, and constitute additional evidence for the protoplasmic-streaming hypothesis of translocation of solutes in plants (17). According to this hypothesis, solute movement is brought about by living protoplasm in which solutes are carried along through sieve tube elements (18).

SUMMARY

Evidence for the independent movement of 4 sap-transmissible viruses was obtained. The viruses of tobacco-mosaic, tobacco-ring spot, cucumber-mosaic and potato X moved out of tobacco or Scotia bean leaves during a period when sugars were moving into the leaves. Leaves were starch-free when inoculated, and subsequently were kept in the dark. In such leaves, starch accumulated along the midribs and veins above scalded stems or petioles. Darkening of inoculated leaves did not affect the velocity of virus translocation. However, because of decreased rates of multiplication in such inoculated leaves, darkening indirectly influenced the development of systemic symptoms by affecting the quantity of virus moved to noninoculated tissues.

When an upper and a lower starch-free leaf of a tobacco or *Nicotiana glutinosa* plant were inoculated, each with a different virus, both viruses became systemic and could be recovered from each inoculated leaf, regardless of dark treatments of inoculated leaves.

Experimental results support the hypothesis of independent translocation of sap-transmissible viruses, and are best explained on the assumption that the translocation mechanism normally operating in plants provides for bi-directional flow of materials in the phloem.

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THE EPIDEMIOLOGY OF XANTHOMONAS TRANSLUCENS (J. J. AND R.) DOWSON ON CEREALS AND GRASSES [1]

M. G. Boosalis [2]

Xanthomonas streak [3] disease of wheat, barley, rye, timothy, brome grass (*Bromus inermis* Leyss.) and quack grass (*Agropyron repens* (L.)) is caused by several varieties of *Xanthomonas translucens* (J. J. and R.) Dowson (6, 15). The disease sometimes becomes epidemic and causes considerable damage to wheat, barley, and brome grass, but it has been difficult to induce epidemics artificially in greenhouse and field. The factors that affect the natural spread of the pathogen and directly or indirectly affect the epidemiology of the disease were investigated.

METHODS.—Lack of a reliable technique for inoculating seedlings of cereals and grasses with *Xanthomonas translucens* is perhaps one reason for the many conflicting reports regarding many phases of the disease. Conventional inoculation methods, such as spraying, injection with hypodermic needle, and various types of wounding, were found inadequate for inoculating a large number of seedlings quickly and effectively. The partial vacuum technique (2) was developed and used in the experiments reported in this paper.

Because of its efficiency, the vacuum technique of inoculation was adopted for detecting and isolating the pathogen from any material suspected of harboring the organism. By this means, the pathogenic bacteria were isolated from infested, sterilized soil that had been stored in the field during the winter. About 30 gm. of infested soil was placed in 3 liters of tap water and allowed to soak for approximately 10 hr. The water was then decanted and poured into a pressure cooker containing seedlings of wheat susceptible to the pathogen. The pressure cooker was then evacuated and the inoculated seedlings were incubated in the greenhouse. Disease symptoms appeared about 5 days later.

The causal organism can also be isolated relatively easily from diseased tissue by the same method. About 100 gm. of the tissue is cut into pieces less than 0.5 cm. long and soaked in water for about 24 hr., then removed. The bacterial suspension is used to inoculate susceptible seedlings as described previously. The host is used for selective isolation of the pathogen from other miscellaneous saprophytic bacteria which are also present in the soil and in the diseased tissue.

Single-colony isolates of the pathogen are finally made from dilution plates prepared from a water suspension of the bacteria obtained by macerating 5 to 7 translucent lesions from the diseased leaves of the vacuum-inoculated seedling in 10 ml. of sterile, distilled water. A final dilution of 1 to 10 million was used for making dilution plates.

MATERIALS.—During the investigation, from 1948 to 1951, isolates of *Xanthomonas translucens* were obtained from diseased tissue of several hosts grown at various localities in Minnesota. Many isolates were made while Xanthomonas streak was epidemic on wheat, barley, and brome grass in the Red River Valley of Minnesota in 1949. Included with the isolates listed in Table 1 was W-4, *X. translucens* var. *undulosa* obtained from Dr. W. A. F. Hagborg in 1948. All the isolates listed were maintained on dextrose-agar slants at approximately 26° C.

The comparative morphologic, cultural and biochemical characters of the varieties of *X. translucens* listed in Table 2 were found to be similar to those reported by Hagborg (6, 7) Wallin (15) and Starr (14).

Varieties of *X. translucens* were differentiated on the

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[3] The common name of Xanthomonas streak is used throughout the paper for the disease of cereals and grasses caused by *Xanthomonas translucens*.

lucens can survive the winter on seeds of wheat and barley. Naturally infected seed of Timstein and Rival wheat and of Mars barley and disease-free seed of each were used in the experiments.

About 500 healthy seeds and 500 naturally infected seeds of each variety of wheat and barley were planted separately in sterilized or nonsterilized soil and germinated in a greenhouse at about 80° F. Barley plants from diseased seeds planted in sterilized and nonsterilized field soil were the only ones infected with *X. translucens*. About 6 per cent of the barley plants grown in the sterilized soil and 3 per cent of those grown in the nonsterilized soil became infected.

Naturally infected and noninfected seeds of Timstein and Rival wheats and Mars barley were also planted in the field. The results were comparable with those from the greenhouse experiments. About 3 per cent of the total infected seeds of barley that germinated gave rise to plants with Xanthomonas streak, while no infected plants resulted from seeds of wheat. Dilution plate counts showed that the viable pathogenic bacterial count on the seed of barley was about 90 per cent greater than that on seed of wheat. The explanation for the higher percentage of bacteria on diseased seed of barley remaining viable is that the glumes may provide some protection to the pathogen against organisms antibiotic to it (5).

Survival on perennial hosts.—In 1948-1949, the overwintering of *X.* translucens on naturally and heavily infected quack grass was investigated at Rosemount Research Center, Minnesota. Bacteria isolated from the diseased leaves were identified as *X. translucens* var. *cerealis*. The pathogenic bacteria were isolated from dead leaves of diseased plants collected on October 15, 1948, November 10, 1948, March 2, 1949, and April 10, 1949. During the investigation, it was observed that many of the newly-developed leaves that were in contact with the diseased leaves developed symptoms of Xanthomonas streak. The results indicate that *X. translucens* can overwinter on naturally-infected leaves of quack grass and that the surviving bacteria can infect the newly formed shoots of the host. Results from our studies also corroborate Wallin's (5) report stating that *X. translucens* overwinters on infected leaves and stems of brome grass.

X. translucens was shown to overwinter on Black Hawk winter wheat, Imperial winter rye and quack grass that were inoculated with pure cultures of the pathogen. The seedlings of the above plants were inoculated with a mixture of isolates Br-1 and Q-1 of *X. translucens* var. *cerealis*, isolates W-1 and W-4 of X. *translucens* var. *undulosa* and isolate Ba-1 of *X. translucens* var. *hordei-avenae*. The noninoculated seedlings (control) were evacuated in 5000 ml. of tap water containing 10 ml. of sterile potato-dextrose broth. About 600 inoculated and noninoculated seedlings of each of the above hosts were transplanted in separate plots in the field. Between June 5 and June 13 water-soaked, translucent lesions appeared on lower leaves of the 3 hosts which had been inoculated the preceding fall. Symptoms of Xanthomonas streak were not noted on any of the control plants. Pathogenic bacteria were isolated on June 7 from the diseased leaves of the 1 perennial and 2 winter annual hosts and identified as *X. translucens* var. *cerealis* and var. *undulosa*. *X. translucens* was not found on leaves of the plants that had not been previously inoculated with the pathogen.

These results prove that *X. translucens* can overwinter on the inoculated leaves of Black Hawk winter wheat, Imperial winter rye, and quack grass, and then infect the newly-formed leaves of the hosts. Under favorable environmental conditions, the diseased perennial and winter annual hosts could become the focal points from which an epidemic of Xanthomonas streak might develop.

In Minnesota, *Xanthomonas translucens* can survive from year to year on kernels of wheat and barley, on wheat straw, and on brome grass, winter wheat, winter rye, and quack grass. These materials, except the diseased kernels of Timstein and Rival wheat, which harbor the pathogen through the winter, may also be the source which produces the initial inoculum. Since brome grass and quack grass are abundant in certain areas and are among the first plants to become green in the spring, there is an opportunity for *X. translucens* to spread from these grasses to cereals and other hosts.

TRANSMISSION OF XANTHOMONAS TRANSLUCENS.—Jones et al. (10) reported that the long-range dissemination of Xanthomonas of barley was through infected seed, and that water is apparently the chief agent of local dissemination, although thrips and aphids may possibly play a considerable part. It was also reported that bacterial exudate from blighted tissue of barley was readily distributed by rain and dew, and to a lesser extent possibly by visiting insects (10). Smith (12) proved that *X. translucens* can be in and on the wheat grains. He (13) also stated that the Xanthomonas-streak bacteria of barley are seed borne. He suggested (12, 13) that the wheat organism could be disseminated with infected straw or by insects and birds. Bamberg (1) did not find infected wheat seedlings in the field or in the greenhouse from seed naturally infected with *X. translucens*.

Transmission by seed.—The preceding experiments show that *X. translucens* in Minnesota remains alive and infectious from year to year on infected seeds of barley. While the pathogen may remain alive on seeds of wheat, the resulting plants are free of Xanthomonas streak. Obviously, then, *X. translucens* may be carried to new localities on barley; and its long range dissemination is thus accounted for.

Transmission by straw.—The studies on the overwintering of Xanthomonas streak revealed that the pathogen remains alive and infectious on infested stubble and refuse of wheat and brome grass. This suggests that the pathogen may be disseminated in the infested straw or chaff which could be blown long distances by the wind.

To test this hypothesis, a mixture of equal amounts

of straw from naturally infected wheat and brome grass was dried and ground to fragments about 0.5 mm. in size. This inoculum was shaken over the seedlings of Timstein wheat and Fisher brome grass grown on folded strips of paper as described by Boosalis (2). *X. translucens* developed only on the leaves of Timstein wheat and Fisher brome grass that had been water-congested and inoculated with the infested straw. About 6 per cent of the plants of Fisher brome grass and about 4 per cent of the Timstein plants became diseased. It appears that *X. translucens* can be disseminated in nature on infested straw of wheat and brome grass, which can be blown considerable distances by the wind. Conditions which permit water-congestion of tissue certainly favor infection by the bacteria from straw.

Transmission by Aphids.—In 1949 there was a heavy infestation of aphids on wheat and barley at St. Paul. Many plants on which the insects were feeding were also severely infected with *X. translucens*. Aphids were seen feeding on areas of the leaf that had developed symptoms of Xanthomonas streak. The insects also frequently moved freely over the affected areas of the leaf that were studded with bacterial exudate, and it seemed, therefore, that they might be vectors.

Aphids were collected from infected plants of wheat and barley, placed in an Erlenmeyer flask containing water, and shaken on a mechanical shaker for about 10 hr. The bacterial suspension obtained from the aphids was then transferred to a pressure cooker filled with 2000 ml. of tap water. Seedlings of Timstein wheat and Mars barley were placed in the pressure cooker and inoculated by the partial-vacuum method. The control treatment consisted of evacuating the seedlings of wheat and barley in tap water.

Approximately 8 per cent of the Mars plants and 5 per cent of the Timstein plants became infected with the pathogen, while all control plants remained healthy. This indicates that aphids may become contaminated with *X. translucens* when visiting diseased plants of wheat and barley. The question now was whether contaminated aphids could effectively transmit the disease to healthy plants.

Aphids collected from infected plants of wheat and barley and transferred to healthy ones in cages did not induce the disease, either in the greenhouse or in the field. Attempts were then made to find out whether the aphids could transmit the disease to water-congested plants. Seedlings of wheat and barley were water-congested and transplanted to pots filled with sterilized soil and then placed in insect cages. The seedlings in one cage were infested with aphids collected from diseased plants of wheat and barley. Aphids collected from healthy plants of wheat and barley were transferred to the water-congested seedlings in a second cage. Contaminated aphids were transferred to the third cage which contained seedlings that were not water-congested. The fourth cage contained only the water-congested seedlings of wheat and barley. The 4 cages were then placed in a low area in the field that had been irrigated. This was done in the evening to prevent the water-congested tissue from drying out. The plants were kept in the cages for 10 days, were then removed and transplanted in the field.

Xanthomonas streak developed on the leaves of wheat and barley that had been water-congested and infested with contaminated aphids. About 5 per cent of the barley plants and 3 per cent of the wheat plants became infected. Variable results were obtained when the experiment was replicated 4 times. In 2 experiments none of the wheat and barley plants became infected regardless of treatment; in another experiment about 10 per cent of the barley and less than 1 per cent of the wheat plants that had been water-congested and infested with contaminated aphids succumbed to the disease. It appears that under favorable environmental conditions aphids can transmit *X. translucens* to wheat and barley and that they may aid in long distance dissemination.

Transmission by rain and winds.—During periods of high humidity, abundant exudate is frequently produced on wheat, barley, and bromegrass in the field. Field observations indicated that the exudate can be readily distributed by beating rains and winds; therefore experiments were made to determine whether infection could result on adjacent noninfected plants. Water-congested seedlings of Timstein wheat, Mars barley, and *Bromus inermis* were placed on a bench directly in line with plants whose leaves were studded with bacterial exudate. A spray jet of water under about 50 lb. of pressure was directed into the infected plants at such an angle that many of the droplets of water deflected from the leaves fell on the leaves of the noninfected, water-congested plants. When the leaves of the noninfected plants were thoroughly covered with droplets of water, the plants were placed in a moist chamber for 48 hours. The control plants were sprayed with tap water and similarly treated. Immediately after the plants were removed from the humidity chambers, they were transplanted to the field.

About 15 per cent of the Mars plants, 10 per cent of the brome grass plants and 8 per cent of Timstein plants that had been water-congested and inoculated with bacterial exudate carried by a driving spray of water became infected with *X. translucens*. No symptoms were observed on any of the control plants. It is concluded therefore that bacterial exudate of *X. translucens* may be disseminated effectively in the field by driving rains and wind. The likelihood of infection is probably increased by environmental conditions which produce water-congested tissue, such as are likely to occur during rainy periods in the summer.

Transmission by contact.—Seedlings of Timstein wheat, Mars barley, and brome grass were inoculated with the variety *cerealis* and placed in a greenhouse kept at about 85° F. When the incipient symptoms appeared on the infected leaves, healthy, water-congested seedlings of the above varieties of cereals and brome grass were placed adjacent to the infected plants in such a way that many of the diseased and healthy leaves were in contact.

TABLE 1.—*Source of isolates of Xanthomonas translucens*

Isolate	Original Host [a]	Year of Collection	Locality
W-1	Wheat	1949	Red River Valley, Minn.
Ba-1	Barley	do	do
Br-1	Brome	do	do
R-1	Rye	do	St. Paul, Minn.
Q-1	Quackgrass	1948	Rosemount, Minn.
W-4	Wheat	1946	Manitoba, Canada

[a] W-4 was a reisolate of Hagborg's *X. translucens* var. *undulosa;* all other isolates were obtained from translucent streaks on the host leaf blades.

basis of pathogenicity on 7 crop plants (Table 2). Five varieties of wheat, 2 strains of *Bromus inermis*, 4 varieties of oats, 2 varieties of barley, 1 variety each of rye and soybean, and *Agropyron repens* were inoculated with 6 isolates of *X. translucens* and 1 isolate of *Pseudomonas glycinea* Coerper, the causal agent of bacterial blight of soybeans. *P. glycinea*, which is nonpathogenic to cereals and grasses used in this study, was included in the control treatment to determine whether or not the resistance of the host was broken down by the severity of the partial-vacuum technique. The criterion of infection for this and subsequent experiments was the development of water-congested, translucent, elongated lesions on leaves of the inoculated plants. Rye isolates were more virulent on rye than on wheat but wheat isolates were equally virulent on wheat and rye.

The 6 isolates of *X. translucens* from cereals and grasses were differentiated into 3 varieties (Table 2). The varieties *undulosa* and *hordei-avenae* are comparable to those in Hagborg's classification (6). The variety *cerealis* is similar to Wallin's (15) new description and change of rank of this variety, except that Wallin's (15) description might be amended to include *Agropyron repens* as a natural host. Hagborg's (6) varieties *hordei* and *secalis* were not isolated from any of the diseased hosts listed in Table 1. Isolates of the 3 varieties of *X. translucens* listed in Table 2 were used in subsequent experiments.

SYMPTOMS OF *X. translucens* ON WHEAT.—Knowledge concerning variability in symptoms produced by *X. translucens* is prerequisite to recognition of the disease and to the understanding of its epidemiology. Special attention was therefore given to the variable symptoms on wheat.

The symptoms of Xanthomonas streak on wheat vary with the environment, the variety, and probably with the severity of infection by root-rot and leaf-spot fungi. Symptoms on heads of wheat seem especially variable, but dark brown to black stripes on the glumes are quite characteristic. Hagborg (7) recently reported that "in varieties of durum wheat (*Triticum durum* Desf.), the glume discoloration is light brown and diffuse rather than dark brown and sharply delimited as in common wheats (*T. vulgare* = *T. aestivum* L.)."

The writer noticed a third symptom on the heads of the variety Timstein and on some of its hybrids. A diffused, translucent, water-soaked discoloration is produced on the glumes in contrast to a light brown or dark brown to black discoloration as described above. In some instances, only a portion of the head is infected; in other instances, the entire head is involved. The diseased heads of wheat remain green much longer than the healthy heads, and, in general, mature 7-10 days later. The bacteria isolated from the diseased glumes were identified as *X. translucens* var. *undulosa* (Table 2, isolate W-1). The symptom described above was produced on heads of the variety Timstein derived from seedlings that were inoculated with isolate W-3 of var. *undulosa* and transplanted in the field. Seeds from the diseased heads of Timstein were severely shrivelled, and on the basis of kernel weight per 100 heads, weighed only about ⅓ as much as healthy seeds.

Since Timestein is being used as a parent in breeding for stem rust resistance, it is important to recognize this new symptom of Xanthomonas streak in order to select not only for rust resistance but also for resistance to streak.

MODE OF OVERWINTERING OF *Xanthomonas Translucens.*—Smith (11) concluded that this pathogen is carried from year to year on wheat seed. His conclu-

TABLE 2. *Degree of infection caused by 3 varieties of Xanthomonas translucens on 14 cereal and grass hosts inoculated by the partial-vacuum method in the greenhouse (40 plants of each inoculated).*

	Degree of infection [a] with isolates of varieties of *X. translucens*						
		Undulosa		Hordei-avenae	Cerealis		Control [b]
Host	W-1	W-4	R-1	Ba-1	Br-1	Q-1	S-1
Wheat							
Newthatch	2	2	0	0	2	2	0
Mida	2	2	0	0	2	3	0
Timstein	3	3	1	0	3	3	0
Pilot	3	3	1	0	3	2	0
Bromus inermis							
var. Fisher	2	0	0	0	3	2	0
var. Parkland	2	0	0	0	3	0	0
Oats							
Marion	0	0	0	0	1	0	0
Mindo	V	0	0	0	0	0	0
Bonda	V	V	V	1	0	2	0
Andrew	V	0	V	0	2	2	0
Barley							
Mars	3	3	2	3	3	3	0
Moore	3	3	2	3	2	2	0
Quackgrass	2	0	0	0	0	3	0
Imperial Rye	2	2	2	0	2	2	0
Habaro Soybeans	0	0	0	0	0	0	2

[a] 0—no infection or a white, yellow, or brown flecking. V—variable infection (immune and resistant reactions). 3—severe infection. 2—moderate infection. 1—slight infection (susceptible reaction).

[b] S-1—*Pseudomonas glycinea* (control).

sion was based on circumstantial evidence since no experiments were made to determine whether seeds from infected plants actually transmitted the disease to the emerging seedlings. Braun (3) presented more conclusive evidence that seed from diseased plants of wheat produced infected seedlings. Bamberg (1) was not able to get diseased plants from infected seed and therefore concluded that *X. translucens* of wheat does not overwinter on the seed, but in the soil. His conclusion, however, was based on experiments in which sterilized soil was infested with a pure culture of the organism and exposed to freezing temperatures without any apparent injury to the pathogen. More recently, Wallin (15) showed that Xanthomonas streak of wheat overwinters on perennial hosts such as *Bromus inermis* and timothy. Jones et al. (10) believed that the organism overwintered on infected seeds of barley.

Experiments were made to determine whether *X. translucens* can survive the winters of Minnesota in infected straw, on seed, in soil, and on perennial hosts. When any of the above habitats were found to harbor the organism through the winter, further studies were made to determine if the diseased material provided sufficient inoculum to cause initial infection in the spring.

Five series of treatments comprising 2 flats each containing 1 lb. of infested straw and 2 flats each containing 1 lb. of noninfested straw were treated as follows: 1) The infested and noninfested straw in the 2 flats was mixed with nonsterilized field soil obtained from the wheat-disease nursery. The infested and noninfested straw in the other 2 flats was mixed with the same type of soil as above except that it was sterilized on 2 consecutive days for 2 hr. each day at 15 lbs. pressure; 2) Similar to above except that the straw was placed on top of the soil; 3) Similar to No. 1 except that sand was used instead of field soil; 4) The straw was placed on top of the sterilized and nonsterilized sand; 5) Flats filled with sterilized and nonsterilized field soil served as the control. The 5 series of treatments were replicated twice, with 1 replicate buried in a wheat field to the level of the flat and the second replicate stored in a room kept at about 27° C.

By the partial-vacuum technique, it was shown that Xanthomonas streak developed on about 50 per cent of the seedlings of Rival wheat inoculated with the bacterial suspension prepared from the infested straw used in the treatments. Most of the infected leaves had 3-4 water-soaked, translucent lesions, but none of the leaves had more than 5. The infective, bacterial population contained in the infested straw was designated arbitrarily as "large" (L.). Subsequent bacterial populations were designed as "medium" (M.) and "small" (S.) when 20 and 10 per cent, respectively, of the inoculated seedlings became infected.

The following May, the flats stored in the field and at 27° C. for 8 mo. were brought to the laboratory and isolations by the partial vacuum technique were made to determine whether the pathogenic bacteria had survived the winter in the infested straw. *X. translucens* overwintered in infested wheat straw kept in the field and in a room at about 27° C. The pathogen remained viable in the infested straw that was mixed with sterilized and nonsterilized field soil and sand. The greatest population of infective bacteria (M.) was found in the infested straw which was placed on top of the above soils. There was no appreciable difference in the population of bacteria on the straw that was stored indoors or in the field. *X. translucens* was not isolated from the control.

To determine whether the surviving bacteria in the infested, overwintered straw could infect the emerging plants of wheat, seeds of Rival wheat were planted in pots filled with the proper straw-infested soils listed in the previous experiment. From planting time until the plants had formed their second leaves the pots were kept in the greenhouse during the day and in humidity chambers at night. Streak appeared on 5 to 7 per cent of the plants resulting from seed planted in the sterilized and nonsterilized field soil and sand which was infested with straw from diseased plants that had overwintered on top of the soil. Only about 1 or 2 per cent of the plants from seeds planted in the overwintered soil types mixed with the infested straw became infected. There was no infection on plants from seed planted in soil containing straw from clean plants. The infested straw apparently harbors viable inoculum from season to season and this inoculum can cause initial infection in the field. The relative number of viable bacteria in the infested, overwintered straw was reduced when this straw was covered with soil.

Survival in the soil.—In 1949, experiments were made to determine whether *Xanthomonas translucens* persists in the soil of fields cropped with wheat heavily infected with the pathogen. During September samples of soil were taken from fields in which wheat had been heavily infected at St. Paul and the Northwest Experiment Station at Crookston, Minnesota. Samples from the same area also were taken from disease-free fields. Seedlings of Rival wheat were inoculated with a water suspension prepared from each of the soil samples and as a check with a water suspension of sterilized soil to which *X. translucens* had been added a few hours prior to inoculation.

None of the seedlings inoculated with the water suspension from the 4 collections of soil became infected, but about 90 per cent of the plants inoculated with the suspension from the inoculated soil became infected. It appeared from this study that the soil from wheat fields heavily infected with *X. translucens* does not harbor infective organisms when freed of diseased plant parts.

Further studies showed that isolates of *X. translucens* variety *undulosa* and 1 isolate of variety *cerealis* were not able to survive long in 4 collections of soil kept at 27° C. and in the field during the winter months unless the soil was sterile and asepsis was maintained.

Survival on seed of barley and wheat.—Studies also were made to determine whether *Xanthomonas trans-*

lucens can survive the winter on seeds of wheat and barley. Naturally infected seed of Timstein and Rival wheat and of Mars barley and disease-free seed of each were used in the experiments.

About 500 healthy seeds and 500 naturally infected seeds of each variety of wheat and barley were planted separately in sterilized or nonsterilized soil and germinated in a greenhouse at about 80° F. Barley plants from diseased seeds planted in sterilized and nonsterilized field soil were the only ones infected with *X. translucens*. About 6 per cent of the barley plants grown in the sterilized soil and 3 per cent of those grown in the nonsterilized soil became infected.

Naturally infected and noninfected seeds of Timstein and Rival wheats and Mars barley were also planted in the field. The results were comparable with those from the greenhouse experiments. About 3 per cent of the total infected seeds of barley that germinated gave rise to plants with Xanthomonas streak, while no infected plants resulted from seeds of wheat. Dilution plate counts showed that the viable pathogenic bacterial count on the seed of barley was about 90 per cent greater than that on seed of wheat. The explanation for the higher percentage of bacteria on diseased seed of barley remaining viable is that the glumes may provide some protection to the pathogen against organisms antibiotic to it (5).

Survival on perennial hosts.—In 1948-1949, the overwintering of *X.* translucens on naturally and heavily infected quack grass was investigated at Rosemount Research Center, Minnesota. Bacteria isolated from the diseased leaves were identified as *X. translucens* var. *cerealis*. The pathogenic bacteria were isolated from dead leaves of diseased plants collected on October 15, 1948, November 10, 1948, March 2, 1949, and April 10, 1949. During the investigation, it was observed that many of the newly-developed leaves that were in contact with the diseased leaves developed symptoms of Xanthomonas streak. The results indicate that *X. translucens* can overwinter on naturally-infected leaves of quack grass and that the surviving bacteria can infect the newly formed shoots of the host. Results from our studies also corroborate Wallin's (5) report stating that *X. translucens* overwinters on infected leaves and stems of brome grass.

X. translucens was shown to overwinter on Black Hawk winter wheat, Imperial winter rye and quack grass that were inoculated with pure cultures of the pathogen. The seedlings of the above plants were inoculated with a mixture of isolates Br-1 and Q-1 of *X. translucens* var. *cerealis*, isolates W-1 and W-4 of X. *translucens* var. *undulosa* and isolate Ba-1 of *X. translucens* var. *hordei-avenae*. The noninoculated seedlings (control) were evacuated in 5000 ml. of tap water containing 10 ml. of sterile potato-dextrose broth. About 600 inoculated and noninoculated seedlings of each of the above hosts were transplanted in separate plots in the field. Between June 5 and June 13 water-soaked, translucent lesions appeared on lower leaves of the 3 hosts which had been inoculated the preceding fall. Symptoms of Xanthomonas streak were not noted on any of the control plants. Pathogenic bacteria were isolated on June 7 from the diseased leaves of the 1 perennial and 2 winter annual hosts and identified as *X. translucens* var. *cerealis* and var. *undulosa*. *X. translucens* was not found on leaves of the plants that had not been previously inoculated with the pathogen.

These results prove that *X. translucens* can overwinter on the inoculated leaves of Black Hawk winter wheat, Imperial winter rye, and quack grass, and then infect the newly-formed leaves of the hosts. Under favorable environmental conditions, the diseased perennial and winter annual hosts could become the focal points from which an epidemic of Xanthomonas streak might develop.

In Minnesota, *Xanthomonas translucens* can survive from year to year on kernels of wheat and barley, on wheat straw, and on brome grass, winter wheat, winter rye, and quack grass. These materials, except the diseased kernels of Timstein and Rival wheat, which harbor the pathogen through the winter, may also be the source which produces the initial inoculum. Since brome grass and quack grass are abundant in certain areas and are among the first plants to become green in the spring, there is an opportunity for *X. translucens* to spread from these grasses to cereals and other hosts.

Transmission of xanthomonas translucens.—Jones et al. (10) reported that the long-range dissemination of Xanthomonas of barley was through infected seed, and that water is apparently the chief agent of local dissemination, although thrips and aphids may possibly play a considerable part. It was also reported that bacterial exudate from blighted tissue of barley was readily distributed by rain and dew, and to a lesser extent possibly by visiting insects (10). Smith (12) proved that *X. translucens* can be in and on the wheat grains. He (13) also stated that the Xanthomonas-streak bacteria of barley are seed borne. He suggested (12, 13) that the wheat organism could be disseminated with infected straw or by insects and birds. Bamberg (1) did not find infected wheat seedlings in the field or in the greenhouse from seed naturally infected with *X. translucens*.

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Aphids collected from infected plants of wheat and barley and transferred to healthy ones in cages did not induce the disease, either in the greenhouse or in the field. Attempts were then made to find out whether the aphids could transmit the disease to water-congested plants. Seedlings of wheat and barley were water-congested and transplanted to pots filled with sterilized soil and then placed in insect cages. The seedlings in one cage were infested with aphids collected from diseased plants of wheat and barley. Aphids collected from healthy plants of wheat and barley were transferred to the water-congested seedlings in a second cage. Contaminated aphids were transferred to the third cage which contained seedlings that were not water-congested. The fourth cage contained only the water-congested seedlings of wheat and barley. The 4 cages were then placed in a low area in the field that had been irrigated. This was done in the evening to prevent the water-congested tissue from drying out. The plants were kept in the cages for 10 days, were then removed and transplanted in the field.

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Transmission by contact.—Seedlings of Timstein wheat, Mars barley, and brome grass were inoculated with the variety *cerealis* and placed in a greenhouse kept at about 85° F. When the incipient symptoms appeared on the infected leaves, healthy, water-congested seedlings of the above varieties of cereals and brome grass were placed adjacent to the infected plants in such a way that many of the diseased and healthy leaves were in contact.

Symptoms of Xanthomonas streak appeared on many of the leaves that had been in contact with the diseased leaves; the percentage of infected leaves was about 20 per cent for Mars barley, 10 per cent for brome grass, and 5 per cent for Timstein. Contact between infected and healthy leaves occurs frequently in nature and undoubtedly results in spread of the disease.

ROOT ROT AND LEAF SPOT FUNGI IN RELATION TO X. TRANSLUCENS OF CEREALS.—During the 1949 epidemic of Xanthomonas streak in the Red River Valley of Minnesota, a high percentage of the wheat and barley plants infected with root rots were also infected with *Xanthomonas translucens* and many of the wheat and barley plants free of root rots were relatively free from Xanthomonas streak. A similar observation was made on winter wheat grown in central Nebraska in 1951. These observations suggested that root rots might predispose cereals to Xanthomonas streak. It was also postulated that the initial infection of *X. translucens* on the plants of wheat and barley was caused by the pathogenic bacteria in association with the host invading fungi. Experiments were made, therefore, to determine whether or not root rot fungi play a part in Xanthomonas streak of wheat and barley and rye.

Laboratory and greenhouse experiments on the relation of root rot and leaf spot fungi to Xanthomonas streak of cereals.—Two isolates of Helminthosporium and 3 isolates of *Xanthomonas translucens* were used in these studies. The isolates of Helminthosporium designated as H–1 and H–2 were obtained from diseased roots of barley and wheat, respectively. Isolate H–1 was identified as *Helminthosporium sativum* but for isolate H–2 no species identification was made. Pathogenicity tests showed that isolates H–1 and H–2 were moderately pathogenic on barley and wheat. Isolate H–1 caused pre-emergence and post-emergence damping-off and spot blotch of Mars and Moore barley. Pre-emergence and post-emergence damping-off of Rival, Newthatch, Timstein, and Mida wheat was produced by isolate H–2. The three isolates of *X. translucens* selected were W–1, Ba–1, and R–1 (Table 2). Experiments were made in the greenhouse in the sterilized and nonsterilized field soil. Seeds of Rival wheat, Imperial winter rye, and Mars barley were inoculated with the proper individual isolates of Helminthosporium or *X. translucens* and with a combination of these 2 organisms grown together (Table 3). The isolate of *X. translucens* used for each cereal is listed in Table 3 under 2 types of inoculum because it was grown on a grain medium and in potato-dextrose broth. The other 2 types of inoculum were increased on a grain medium. Noninfested potato-dextrose broth and noninfested grain were used for the control.

The following procedure was used in increasing the inoculum. The grain medium was inoculated with an isolate of Helminthosporium 8 days before the bacterial isolate was added. The fungus and bacterial inoculum was then allowed to grow for 14 days before it was used to inoculate the seeds. The inoculum containing only an isolate of Helminthosporium was grown for 22 days; and the inoculum containing an isolate of *X. translucens* was grown 4 days. Twenty-four-hour-old cultures of the 3 bacterial isolates were also grown separately in sterilized, 250 ml. Erlenmeyer flasks, each filled with 100 ml. of potato-dextrose broth.

TABLE 3.—*The percentage of plants of 3 cereals infected with Xanthomonas translucens resulting from seed inoculated with Helminthosporium sp.; X. transulcens and with both Helminthosporium sp. and X. translucens grown on a grain medium*

Host	Organism and percentage of plants infected with *X. translucens* [a]		
	Helminthosporium sp.	*X. translucens*	*Helm.* sp. & *X. translucens*
Rival wheat	0	2	15
Mars barley	0	3	30
Imperial rye	0	2	20

[a] Rival wheat was inoculated with isolates H-2 (*Helminthosporium* sp.) and W-1 (*X. translucens* var. *undulosa*); Mars barley, with isolates H-1 (*Helminthosporium sativum*) and Ba-1 (*X. translucens* var. *hordei-avenae*); Imperial rye, with isolates H-1 (*Helminthosporium sativum*) and R-1 (*X. translucens* var. *undulosa*). Perfect emergence would be 200. Percentage of diseased plants was based on total number of plants emerging, ranging from 150 to 190 plants.

About 200 seeds of each cereal were inoculated with one of the types of inoculum listed in Table 3, and planted 3.0 inches deep in 10 6-in. pots filled with sterilized field soil. The pots for each of the cereals were randomized in a greenhouse maintained at about 85° F., and watered daily with tap water.

A high percentage of the plants of wheat, barley, and rye resulting from seeds inoculated with both *Helminthosporium* and *X. translucens* were infected with *X. translucens*. Spot blotch also developed on a high percentage of the plants of Mars barley. As soon as the symptoms of the disease appeared most of the infected plants were extirpated to prevent spread of the pathogen to adjacent noninfected plants. Table 3 shows that 30 per cent of the plants of Mars barley, 20 per cent of the plants of Imperial rye, and 15 per cent of the plants of Rival wheat derived from seed inoculated with both Helminthosporium and Xanthomonas developed symptoms of Xanthomonas streak. About 3 per cent of the Moore barley and 2 per cent of the Imperial rye and Rival wheat resulting from seed inoculated with *X. translucens* increased on the grain medium or on potato-dextrose broth were infected with the pathogenic bacteria. The seeds of the 3 cereals inoculated with *Helminthosporium* sp. did not give rise to plants infected with *X. translucens*. Plants from the control treated seed of the 3 cereals were free of symptoms caused by *X. translucens*.

This experiment was replicated in nonsterilized field soil, and results again were comparable to the preceding results. The greenhouse studies show that root rot and leaf spot fungi associated with *X. translucens* increased the incidence of Xanthomonas streak on

barley, wheat, and rye planted in sterilized or non-sterilized field soil.

Field experiments on the relation of root rots to Xanthomonas streak.—Three varieties of wheat, Timstein, Rival, and Lee, 2 varieties of barley, Moore and Mars, and 1 variety of winter rye, Imperial, were used for the field experiments. Seeds of each variety of the 3 cereals were inoculated with 4 types of inoculum as previously described. All varieties of the 3 cereals treated with a comparable type of inoculum were planted in the same block. Each variety, however, was planted in separate, randomized plots within the block. The blocks were randomized and each treatment was replicated twice.

About 100 seeds were planted in each row, making a total of 400 seeds in each plot. One qt. of the inoculum grown on the grain medium was mixed with 1 qt. of field soil and spread on top of the seeds sown in 1 row. Approximately 500 ml. of the bacterial inoculum grown on potato-dextrose broth for 48 hr. was diluted with 2000 ml. of tap water and sprinkled on the seeds planted in 1 row. Sterilized grain and sterilized potato-dextrose broth were applied at the same rate to the controls. The depth of planting after the seeds were covered with field soil was about 3 in.

The only plants infected in the third leaf stage were those resulting from seeds of wheat, barley, and rye inoculated with isolates of *Helminthosporium* sp. and *X. translucens* grown together on the grain medium. The highest incidence of Xanthomonas streak was on Mars barley, which had 40 per cent of its plants infected with the pathogen. The percentage of diseased plants for the other varieties of cereals listed in Table 4 was as follows: Moore barley—37, Imperial rye—30, Timstein wheat—28, Rival wheat—25, and Lee wheat—10.

The symptoms of Xanthomonas streak developed late on the leaves formed after the third leaf, not until flowering time or even when the kernels were in the milk stage. New centers of infection appeared on the leaves of most of the diseased plants at this time. Infection was also evident on the leaves of many adjacent plants which were previously free of the disease. The percentage of diseased plants for each variety of cereal at the milk stage of the kernel development was more than twice the percentage of diseased plants counted at the 3-leaf stage of development (Table 4). For example, 28 per cent of the plants of Timstein wheat were infected during the 3-leaf stage of development, but it was 85 per cent when the kernels of the plants had reached the milk stage. A comparable increase in the percentage of diseased plants was also noted for the other varieties of wheat, barley, and rye listed in Table 4. Plants infected with *X. translucens* were also severely infected with root rot.

When the kernels were in the milk stage, it was observed that a number of plants of Timstein, Rival, and Lee resulting from seeds inoculated with isolate H-2 of *Helminthosporium* sp. were infected with *X. translucens*. Table 4 shows that 40 per cent of the Timstein plants, 25 per cent of the Rival plants and 10 per cent of the Lee plants were infected with the bacterial pathogen. The symptoms of the disease occurred primarily on the leaves, although translucent lesions developed on the glumes of many plants. Roots of the plants infected with *X. translucens* were also severely infected with root rot fungi. About 60 per cent of the fungi isolated from the diseased roots of wheat were identified as *Helminthosporium* sp. of which the cultural characteristics were similar to those of isolate H-2. A relatively low percentage of the plants of all the varieties of wheat, barley, and rye derived from seeds inoculated with *X. translucens* grown on a grain medium or on potato-dextrose broth were found to be infected with the bacteria. In some instances a few of the plants from the control seeds were infected also.

Greenhouse and field experiments showed that a high incidence of Xanthomonas streak occurs on wheat, barley, and rye derived from seed inoculated with a pathogenic root rot isolate of Helminthosporium grown together with an isolate of *X. translucens* on a grain medium. The mechanism by which the root rot isolate grown with the bacterial isolate causes the initial infection on the plants was not investigated. The field experiments also showed that wheat severely infected with root rot fungi was also infected with *X. translucens*. The source and the mode of dissemination of the bacterial inoculum causing Xanthomonas streak on the plants of wheat from seed inoculated

TABLE 4.—*Percentage of plants of wheat, barley, and rye infected with X. translucens when grown from seeds planted in the field and inoculated with Helminthosporium sp., X. translucens, and with both grown together on grain medium.*

Host	Organism and percentage of plants infected with *X. translucens* [a]					
	Helminthosporium sp.		*X. translucens* [b]		*Helm.* sp. & *X. translucens*	
	3-leaf stage	milk stage of grain	3-leaf stage	milk stage of grain	3-leaf stage	milk stage of grain
Wheat:						
Rival	0	25	0	7	25	60
Timstein	0	40	0	5	28	85
Lee	0	10	0	3	10	25
Barley:						
Mars	0	6	0	0	40	80
Moore	0	4	0	2	37	78
Rye:[c]						
Imperial	0	—	0	—	30	—

[a] Wheat was inoculated with isolates H-2 (*Helminthosporium* sp.) and W-1 (*X. translucens* var. *undulosa*); barley, with isolates H-1 (*Helminthosporium sativum*) and Ba-1 (*X. translucens* var. *hordei-avenae*); rye, with isolates H-1 (*Helminthosporuim sativum*) and R-1 (*X. translucens* var. *undulosa*). Perfect emergence would be 200. Percentage of diseased plants was based on total number emerging ranging from 285 to 390.

[b] Similar results were obtained when seeds of the 3 cereals were inoculated with the bacterial pathogen grown on potato-dextrose broth.

[c] The winter variety of rye, Imperial, did not head.

with a root rot fungus was not determined. Although these experiments were made only once, results indicate that root rot fungi may be an important factor in the epidemiology of Xanthomonas streak of wheat, barley, and rye, and possibly other grasses.

Discussion. — Although *Xanthomonas translucens* causes a common and destructive disease of cereals and grasses, previous investigations have been confined primarily to the classifiation and natural host range of the pathogen (1, 4, 6, 15). The present investigations have shown how the pathogen overwinters in Minnesota, and some ways in which it is disseminated. It has also shown that root-rot fungi may predispose cereals to Xanthomonas streak. This information should help in the prevention of the disease by cultural methods and in understanding the ocurrence of epidemics.

The common names given to the diseases caused by *Xanthomonas translucens* do not adequately describe the symptoms. For example, although the name "black-chaff" of wheat is commonly used, it is not truly descriptive. Common names need not necessarily be descriptive, but this name has given the impression that all black discoloration of the head is caused by *X. translucens*. It is believed that "Xanthomonas streak" proposed by Wallin (15) is a more appropriate common name.

It is difficult to make an accurate diagnosis of Xanthomonas streak in the field by studying the symptoms on the head only, because other microorganisms, including fungi and bacteria, as well as environmental factors may produce black discolorations similar to those produced by *X. translucens* (7). The symptoms on the head may also vary with the variety. The most frequent symptom reported on the heads is dark brown to black stripes on the glumes. On durum wheats, however, the glume discoloration is light brown (7); and on Timstein and some of the Timstein derivatives, there is no light brown, dark brown or black discoloration but only a diffused, translucent, water-soaked discoloration of the glumes. Xanthomonas streak can be diagnosed fairly accurately, however, if bacterial exudate in the form of transparent scales or yellow beads is present on some infected portion of the tissue. Microscopic observations and cultural studies are necessary, however, to be certain of the causal agent.

One of the most important reasons for the paucity of information regarding many phases of the epidemiology of Xanthomonas streak is undoubtedly the lack of a technique for detecting the causal agent when present in relatively small numbers. The author therefore devised and adopted, especially for this purpose, the partial-vacuum technique for inoculating seedlings with bacteria. Because of its efficiency, the partial-vacuum technique was used for inoculating simultaneously a large number of seedlings with the same batch of inoculum, for detecting naturally surviving bacteria in small numbers on overwintered material and on possible agents of dissemination, and, in studies not reported here, for inoculating uniformly and heavily many varieties and hybrid lines of wheat to be tested for resistance in the field.

This new technique was also used for water-congesting the host tissue. Such water-congestion apparently is requisite for infection of plants when inoculated by other methods. Water congestion occurs commonly in nature (8, 9) and, in all probability, is important in the effective transmission of *X. translucens* in the field. Although this new technique of inoculation may be somewhat drastic as compared to natural means of inoculation, it served its purpose in elucidating many phases of the epidemiology of *X. translucens*.

Xanthomonas translucens can persist from one season to the next on winter wheat, winter rye, and quack grass; on the straw of wheat and brome grass; and on seeds of barley and wheat. The winter hosts afforded the bacteria the best means for overwintering, and much of the initial inoculum in spring probably comes from these sources. The number of viable bacteria in straw was considerably reduced when the infested straw was mixed with soil. It may be that *B. subtilis* (5), which is antibiotic on artificial media, and other soil-inhabiting microorganisms are deleterious to *X. translucens* in soil and tend to eliminate it through antibiotic action. This could also explain why the pathogenic bacteria do not survive in the soil for any appreciable time. Only a small number of bacteria remain viable on severely infected seed of wheat; consequently, the resulting plants have been found to be free from Xanthomonas streak. Bamberg (1), who also made experiments at Minnesota, reported that plants from diseased seed of wheat were not infected with the pathogen.

The mode of overwintering of *X. translucens* suggests 3 possible sanitary measures for controlling the disease—using clean seed, plowing under crop residue, and destroying perennial weeds susceptible to the pathogen.

Since the pathogenic bacteria overwinter on straw and seed, they may be disseminated long distances on these materials by man or wind. Local dissemination of *Xanthomonas translucens* is by wind-blown rain, contact, and possibly certain insects. In view of the fact that barley refuse may harbor *X. translucens* as well as such leaf spot fungi as *Helminthosporium teres* and *Helminthosporium sativum*, it is also possible that hyphae of these fungi may subsequently carry the bacteria to the emerging seedlings of cereals. Further studies should be made to investigate the possibility that another disseminating agent of the bacterial pathogen may be the spores of the leaf spot fungi which are on plants infected with *X. translucens*.

Experiments have disclosed the existence of a biotic relationship between root rots such as *Helminthosporium sativum* and *Helminthosporium* sp. and *Xanthomonas translucens* which appears to have a profound effect on the epidemiology of Xanthomonas streak of cereals.

Wheat infected with root rot fungi (*Helminthosporium* spp.) apparently is predisposed to the attack of

Xanthomonas translucens. In controlled experiments a greater incidence of natural bacterial infection occurred on plants infected with root rot fungi than on the plants free from root rot and there was a positive association between the incidence of the two diseases in commercial fields of wheat.

Root rot associated with *Xanthomonas translucens* may have a strong influence on the incidence of Xanthomonas streak on the seedlings of barley, wheat, and rye. Seeds of the 3 cereals inoculated with *X. translucens* alone resulted in a few infected seedlings, whereas 25 to 40 per cent of the seedlings from seeds inoculated both with a root rot fungus (*Helminthosporium* sp.) and *X. translucens* succumbed to Xanthomonas streak.

The mechanism by which root rot fungi increase the incidence of Xanthomonas streak was not extensively studied. It is possible that plants infected with root rot fungi may be more subject to water-congestion, which is an important factor for the abundant infection of the host by bacteria. It is also possible that bacteria associated with root-rot fungi are carried to the infection court of the emerging seedlings by the host-invading hyphae. This relationship may help to explain the conflicting reports regarding many phases of the disease and the difficulty encountered in causing artificial epidemics.

SUMMARY

Xanthomonas streak of cereals and grasses caused by several varieties of *Xanthomonas translucens* occurs commonly in Minnesota.

The partial-vacuum technique was devised for inoculating seedlings with bacteria, for isolating the pathogen from materials suspected of harboring the pathogen, and for water-congesting the host tissue. By this means it was possible to determine the mode of overwintering of *Xanthomonas translucens* and possible agents of dissemination.

The isolates of *X. translucens* from cereals and grasses were identified on the basis of pathogenicity on wheat, barley, rye, oats, brome grass, and quack grass as belonging to the varieties *undulosa*, *hordeiavenae*, and *cerealis*.

A new symptom of Xanthomonas streak was noted on the heads of Timstein wheat and some Timstein derivatives, on which a glistening, translucent, diffused, water-soaked appearance of the glumes is characteristic with absence of any appreciable light brown or dark brown to black discoloration.

In Minnesota the pathogenic bacteria may persist from one season to the next on seed of barley and wheat, on straw of barley and brome grass and on winter hosts such as winter wheat, winter rye, quack grass, and brome grass.

With the exception of wheat seed, the above material which harbors the pathogen through the winter, may also provide the initial inoculum which ultimately results in producing the initial infection in the spring.

X. translucens does not survive in non-sterile soil for an appreciable length of time unless it is in or on plant parts.

X. translucens may be transmitted by aphids, straw, wind and rain, by diseased plants coming in contact with healthy plants, and probably by root rot and leaf spot fungi.

Effective transmission by aphids, straw, wind and rain, and contact occurred only when the host tissue was water-congested.

Experimental evidence indicates that root-rot and leaf-spot fungi predispose wheat to attack by *X. translucens*.

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FURTHER STUDIES ON TOMATO CURLY TOP IN BRAZIL [1]

A. S. Costa [2]

Studies on curly top of tomatoes (*Lycopersicon esculentum* Mill.) and tobacco (*Nicotiana tabacum* L.) in Brazil (3) afforded evidence that these diseases are caused by a variety of the sugar beet curly top virus (*Ruga verrucosans* Carsner & Bennett). The new variety was named *brasiliensis* and its transmission by the leafhopper, *Agallia albidula* Uhl., reported previously (11), was confirmed.

The relative difficulty in obtaining infection of tomato plants when inoculated with *Agallia albidula* and the frequent failure in attempts to recover the virus from infected field tomato plants by means of non-viruliferous leafhoppers (3) stimulated further search for other strains of the virus and for other possible vectors. It has since been verified that at least another virus, possibly a variety of sugar beet curly top virus, also occurs in tomato plantings in the State of São Paulo, Brazil. The disease caused by the new virus in tomatoes cannot be distinguished on the basis of symptoms from that caused by the *brasiliensis* variety. It has also been ascertained that the 2 viruses are transmitted by different species of insect vectors.

HOST RANGE OF THE NEW TOMATO CURLY TOP VIRUS.—So far tomato has been the only host plant found naturally affected with the new curly top virus. Comparative inoculation tests with this virus and the *brasiliensis* variety showed that the host range of the former is much narrower than that of the latter. The results are in Table 1. Tomato, currant tomato (*Lycopersicon pimpinellifolium* Mill.), jimson weed, and *Solanum nigrum* were found susceptible to the new virus, but sugar beet plants could not be infected. The first 3 of these plants, and also sugar beets, were infected by the *brasiliensis* variety, but *S. nigrum* was not, thus confirming previous results (3).

DESCRIPTION OF SYMPTOMS CAUSED BY THE NEW TOMATO CURLY TOP VIRUS.—*Tomato.*—The first symptoms shown by inoculated tomato plants appear 1-2 wk. after inoculation, and consist of vein clearing or a slight yellowing along some small veins of partly developed leaflets. They then roll upwards, and their veins become more noticeable than veins of normal leaflets (Fig. 1). Leaves formed after infection show strong rolling of the leaflets and have a tendency to be more upright. The growth of the plant is retarded, and affected plants in the field may or may not die, but plants infected in the greenhouse remain alive.

The virus becomes systemic in tomato plants more rapidly than does the *brasiliensis* variety, but the symptoms caused by both are alike (Fig. 2).

The symptoms shown by infected *Lycopersicon pimpinellifolium* plants are similar similar to those of tomato plants.

Datura stramonium.—Symptoms shown by infected jimson weed appear about 10 days after inoculation, and are of the same nature of those shown by tomato plants. Clearing or yellowing of the small veins is followed by vein thickening and curling of leaves downwards. Small veins become raised on the upper surface of the leaf, and larger veins are somewhat tortuous. Diseased plants become stunted. Symptoms caused by the *brasiliensis* variety are similar to those just described, but are slightly more severe.

Solanum nigrum.—Plants of this species show strong clearing or yellowing of the veins about 8 days after inoculation, followed by curling of leaves, vein thickening, and severe stunting.

TRANSMISSION.—*Mechanical inoculation.*—Attempts to transmit the new variety of tomato curly top virus by mechanical inoculation have been unsuccessful. A total of 370 tomato plants of various ages and 76 jimson weed seedlings were inoculated by rubbing with juice from diseased tomato plants. Carborundum was sprinkled on the leaves of test plants prior to inoculation. Some inoculations were made with inoculum diluted 1:5 in 0.01M sodium sulphite. All inoculated plants remained healthy. A total of 150 tomato seedlings were inoculated by needle punctures, with a group of 5 insect pins fastened together, dipped in the inoculum, and then used to puncture the test plants in at least 5 different places near the growing point. The results were also negative.

Grafting.—Transmission of the new variety of tomato curly top virus to susceptible plants is accomplished easily by means of grafts. It is more readily transmitted by grafting from tomato to tomato than the *brasiliensis* variety.

Attempts to transmit the new virus to tobacco and potato plants (*Solanum tuberosum* L.) by grafting with diseased tomato scions gave negative results, although the scions had established union with the test plants in most cases.

Seed transmission.—More than a thousand tomato seedlings have been grown from seeds of fruits produced by plants infected with the new type of curly top. All plants were healthy.

Specific transmission of two types of tomato curly top virus by different leafhoppers.—Preliminary experiments with the new virus causing tomato curly top showed that it could be transmitted by the leafhopper *Agalliana ensigera* Oman,[3] an insect that has failed in transmitting the *brasiliensis* variety of *Ruga verrucosans* (3). Attempts to transmit the new tomato

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[3] The writer is indebted to P. W. Oman, of the Bureau of Entomology and Plant Quarantine, Agricultural Research Administration, U. S. Department of Agriculture, for identification of this insect.

TABLE 1.—*Comparative tests on the host range of the Agalliana-transmitted virus and the brasiliensis variety of the sugar beet curly top virus*

Plants tested	Inoculations with *Agalliana ensigera* and *Agallia albidula* carrying, respectively: new curly top virus			var. *brasiliensis*		
	Insects per plant	Plants		Insects per plant	Plants	
		Infested	Infected		Infested	Infected
	(No.)	(No.)	(No.)	(No.)	(No.)	(No.)
Beta macrocarpa Guss.	25	8	0	10	8	8
B. patellaris Moq.	10	5	0			
B. patula Ait.	5	6	0	5	6	6
Do.	10	9	0	10	8	7
B. vulgaris L. var. R. & G., Old Type	1	12	0			
Do.	25	52	0	15	27	5
var. *cycla* L.	10	34	0	15	8	0
Brassica pe-tsai Bailey	5	10	0	5	10	1
Do.	10	3	0			
Callistephus chinensis Nees	5	10	0	5	10	0
Capsicum frutescens L.	5	20	0	5	36	3
Do.	25	16	0			
var. *grossum* Bailey	5	20	0	5	28	9
Do.	15	8	0			
Datura stramonium L.	5	29	17	5	28	13
Euphorbia prunifolia Jacq.	20	20	0			
Fagopyrum esculentum Gaertn.	10	97	0	10	14	9
Galinsoga parviflora Cav.	1	2	0	1	2	0
Gossypium hirsutum L.	10	6	0	10	7	0
Helianthus annuus L.	10	25	0			
Lycopersicon pimpinellifolium	5	32	23	5	32	5
Nicandra physaloides Gaertn.	10	16	0	5	16	0
Nicotiana glauca Graham	10	2	0			
N. glutinosa L.	25	2	0	5	2	2
N. tabacum L. (31 varieties)	5	262	0			
×*Petunia hybrida* Hort.	10	26	0	10	16	3
Physalis sp.	10	21	0	10	21	0
Samolus floribundus H.B.K.	5	14	0	5	18	16
Do.	10	5	0			
Solanum melongena L.	5	30	0	5	10	0
S. nigrum L.	1	8	8	10	20	0
Do.	10	23	23	20	3	0
Spinacia oleracea L.	10	5	0	10	5	5
Stellaria media (L.) Cyr.	10	16	0	1	10	8
Zinnia elegans Jacq.	10	5	0	10	5	0

curly top virus by *Agallia albidula* failed, indicating that there was a specific relationship between the 2 viruses and the 2 species of leafhoppers.

A series of tests was started to determine whether the specific transmission already observed was absolute or partial. Tomato and jimson weed were inoculated separately with the 2 varieties of tomato curly top virus, and spiny bur (*Acanthospermum hispidum* DC.) was infected with the *brasiliensis* variety of virus. Non-viruliferous *Agalliana ensigera* and *Agallia albidula* insects were then caged together on each of the infected plants. After the leafhoppers had fed for a few days, the two species were segregated, and groups of 5-10 insects of each species were transferred to caged tomato, jimson weed, and spiny bur seedlings. The results are summarized in Table 2. In no instance was *Agalliana ensigera* able to transmit the variety *brasiliensis*, nor was *Agallia albidula* a vector of the new variety of curly top virus, demonstrating that the specificity of the 2 leafhoppers in the transmission of the 2 viruses was probably absolute. The data also indicated that *Agalliana ensigera* was apparently a more efficient vector of the new tomato curly top virus than was *Agallia albidula* of *brasiliensis*.

Attempts to break the specificity of Agalliana ensigera and Agallia albidula in the transmission of two types of curly top virus.—In tests with races of *Cicadulina mbila* Naude that were either active or inactive in the transmission of corn streak virus (*Fractilinea maidis* (Holmes) McKinney), Storey (13) showed that a number of inactive insects fed on diseased plants could be made infective if their abdomens were punctured with a needle.

Tests were made to determine whether or not the specificity of the 2 leafhoppers in the transmission of the 2 types of tomato curly top viruses could be broken by puncturing the abdomen of the insects. Groups of nonviruliferous insects of the 2 species were fed for a few days on plants infected by the variety of virus that they did not transmit, that is, *Agalliana ensigera* was caged on spiny bur plants infected by *brasiliensis*, and *Agallia albidula* was fed on tomato plants infected

FIG. 1. Symptoms induced by the *Agalliana*-transmitted curly top virus in tomato plants. A, leaf showing yellowing of the small veins (a) in comparison with healthy (b); B, infected tomato plant (a) about 20 days after inoculation and healthy control (b); C, leaf from an infected plant at a more advanced stage, showing strong rolling of leaflets and conspicuous veins (a), and normal leaf.

with the new curly top virus. At the end of the feeding period the insects were anesthetized and their abdomens were each punctured once or twice with a fine entomological pin. After treatment the *Agalliana ensigera* insects were caged on healthy spiny bur seedlings, and the *Agallia albidula* leafhoppers on healthy tomato seedlings. Both were tested in groups of 5 insects per plant. Rate of survival of the treated leaf-

TABLE 2.—*Result of cross-specificity tests with Agalliana ensigera*[a] *and Agallia albidula, and the two viruses that cause tomato curly top*

Source of Virus	Type of Virus	Vector species	Number of test plants infested and infected					
			Tomato		Jimson weed		Spiny bur	
			Infested	Infected	Infested	Infected	Infested	Infected
Tomato	New virus	*Agalliana ensigera*	345	232	16	9	165	0
		Agallia albidula	141	0	15	0	12	0
	Brasiliensis	*Agalliana ensigera*	88	0	12	0	54	0
		Agallia albidula	73	0	8	4	92	35
Jimson weed	New virus	*Agalliana ensigera*	136	50	....	....	....	....
		Agallia albidula	62	0	....	....	12	0
	Brasiliensis	*Agalliana ensigera*	79	0	26	0	37	0
		Agallia albidula	41	0	17	1	46	2
Spiny bur	*Brasiliensis*	*Agalliana ensigera*	228	0	29	0	247	0
		Agallia albidula	108	15	44	19	172	74

[a] A minimum of 3, but usually from 5 to 10, insects were used to infest each test plant. In many tests the same lot of insects was used to infest two batches of plants.

hoppers was high.

In a series of 3 tests, 95 individuals of *Agalliana ensigera* treated in the indicated manner were caged on a total of 68 spiny bur seedlings and gave no transmission. A total of 140 individuals of *Agallia albidula* tested similarly on 104 tomato seedlings also gave negative results.

Tests with other insects.—Several species of insects, especially leafhoppers, were tested occasionally as possible vectors of the new tomato curly top virus. Many tests were made with unidentified species which were not determined later because they had given negative results. Also, no transmission was obtained with the white flies, *Bemisia tabaci* (Genn.) and *Trialeurodes tabaci* Bondar; with the aphids, *Aphis fabae* Scolopi and *Macrosiphum solanifolii* (Ashm.), and with the leafhoppers, *Baldulus maidis* (DeLong & Wolcott) and *Xerophloea viridis* (F.).[4] From all species tried so far only the two species of *Agalliana*, *A. ensigera* and *A. sticticollis* (Stahl) transmitted the virus.

Incidence of the two types of tomato curly top in field plantings.—When tests were started to determine the relative incidence of the 2 types of curly top, tomato plantings made in 3 different locations, at different times, were inspected and 1 sprout each of 10 or 20 tomato plants showing curly top were collected at random from each field. Each sprout, with only 3 or 4 upper leaves, was placed in a test tube with water and confined in an insect cage. Five nonviruliferous leafhoppers of each of the 2 species, *Agalliana ensigera* and *Agallia albidula*, were introduced in each cage. After a feeding period of 2-4 days the two species were segregated. *Agalliana ensigera* leafhoppers were caged on tomato seedlings, and *Agallia albidula* were tested on spiny bur seedlings, which are very susceptible to the *brasiliensis* variety. The use of spiny bur helps to avoid errors due to possible vector mixtures, since it is not susceptible to the *Agalliana*-transmitted virus.

The tomato curly top virus transmitted by *Agalliana ensigera* was more prevalent than the variety *brasiliensis* in the tomato plantings sampled in 1949. Samples tested in 1950 yielded mostly the variety *brasiliensis*. It is probable that in most of the unidentified cases the *brasiliensis* variety was involved because it is more difficult to recover from tomatoes than is the *Agalliana*-transmitted virus. In no case were the 2 viruses recovered from the same plant. Results of tests are in Table 3.

Characteristics, distribution, and host range of the vectors.—*Agalliana ensigera* and *A. sticticollis* are very abundant on many cultivated plants and weeds in the State of São Paulo. Oman (10) recorded *A. ensigera* from Argentina and Bolivia. He also recorded *A. sticticollis* (9, 10) from several states of Brazil, British Guiana, Trinidad, Tobago, St. Vincent, Dominican Republic, Puerto Rico, Cuba, and from Province Missiones, Argentina.

FIG. 2. Tomato plants showing curly top. A, plant infected with the *brasiliensis* strain; B, plant infected with the *Agalliana*-transmitted virus.

[4] The writer is indebted to Miss Louise M. Russell and to P. W. Oman, Bureau of Entomology and Plant Quarantine, Agricultural Research Administration, U. S. Department of Agriculture, for the identification of the white flies and leafhoppers, respectively; to R. C. Dickson, Division of Entomology, University of California, for identification of the aphids.

TABLE 3.—*Results of tests to determine the relative incidence of 2 types of tomato curly top in 3 farms*

Farms where samples were collected	Date of collection	Plants sampled	Number of plants from which the indicated virus was recovered		
			New virus	*Brasiliensis*	Not identified
Mato Dentro	6- 4-1949	10	10	0	0
	6-24-1949	10	9	1	0
	2- 6-1950	6	0	3	3
	10-20-1950	10	3	2	5
Santa Eliza	10- 3-1949	10	9	0	1
	7- 8-1950	5	0	3	2
	8- 4-1950	10	0	2	8
	10-23-1950	10	3	5	2
Sta. Genebra	7-15-1950	37	1	12	24
	8- 9-1950	10	0	2	8
	10-25-1950	20	0	6	14

Agalliana ensigera is slightly smaller than *A. sticticollis*, but the 2 insects look alike and are difficult to tell apart in mixed populations. Differences can be noticed easily, however, when pure populations of the 2 species are compared. Identification of the 2 species is done rather easily by observing the male genitalia. The dorsal spines of *A. ensigera* are saber-like in lateral view (10), whereas *A. sticticollis* has forked spines (9). The writer has been able to identify males of the 2 species by observation of the genitalia, without killing the insects. Anesthetized viruliferous males were examined under the low power microscope; by pressing gently the ends of their abdomens with a needle, the genital structures protruded somewhat, permitting the dorsal spines to be recognized. The identified insects were later caged individually on tomato seedlings, and it was established beyond doubt that both species could act as vectors of the new tomato curly top virus.

Female *Agalliana ensigera* are larger than males and lighter in color. This species has been reared in cages on the following plants: spiny bur, *Atropa belladona* L., *Callistephus chinensis*, *Crotalaria juncea* L., *C. lanceolata* E. Mey., jimson weed, *Nicotiana paniculata* L., *Solanum nigrum*, sunflower, and *Solanum tuberosum*.

In Argentina, *Agalliana ensigera* is the vector of curly top of sugar beet caused by *Ruga verrucosans* C. and B. var. *distans* B.C.C.B. (2). *A. ensigera* does not breed on tomatoes there (2), whereas it could be reared on tomatoes in Brazil.

Agalliana sticticollis does not feed well on tomatoes and apparently does not breed on this plant. This species has been tried less extensively in the transmission of the *Agalliana*-transmitted tomato curly top virus.

Both *Agalliana ensigera* and *A. sticticollis* have been reared with excellent results on sunflower plants, which are easy to raise and support large numbers of insects.

VIRUS-VECTOR RELATIONSHIPS.—*Relation of number of vectors to percentage of transmission.*—In tests to determine the relative efficiency of different numbers of vectors in the transmission of the new tomato curly top virus, fully charged, viruliferous *Agalliana ensigera* insects were caged on tomato seedlings in numbers of 1, 2, 3, and 5 leafhoppers per plant. Results of 3 tests are in Table 4. Data on transmission from several miscellaneous experiments in which varying numbers of vectors per plant were used are also summarized in the table. *A. ensigera* was a very efficient vector of the virus, and single insect inoculation gave about 50 per cent or higher infection. Inoculations with larger number of insects increased transmission, and 5 insects per plant usually gave 100 per cent success.

Incubation period of virus in Agalliana ensigera.—In tests to determine the incubation period of the virus in *Agalliana ensigera*, nonviruliferous insects reared on sunflower plants were fed on diseased tomato plants for varying periods, and then transferred on a series of healthy tomato seedlings at timely intervals. The period of time beginning when the insects started feeding on the source of virus until the first cases of transmission were obtained in the successive transfers was considered as the incubation period. Transmission was obtained in a few cases with insects in which the virus had an incubation period of 24 hr., but not with insects in which the incubation period was 16 hr., thus indicating that the minimum incubation period lies between these limits. Results are in Table 5.

Period of retention in vector.—Tests were made to determine the time fully-charged, viruliferous *Agalliana ensigera* would remain infective without having access to a new source of virus. Male and female adult insects that had fed on diseased tomato plants for at least a week were confined singly in leaf cages (7), and their sexes were determined under the low power microscope. Each leaf cage with 1 insect was then placed on a tomato seedling and transferred daily to

TABLE 4.—*Results of tests in which tomato test plants were inoculated with different numbers of viruliferous leafhoppers, Agalliana ensigera, per plant*

Test number	Number of tomato seedlings inoculated and infected by indicated number of viruliferous leafhoppers							
	1		2		3		5	
	Infested	Infected	Infested	Infected	Infested	Infected	Infested	Infected
1	20	9	20	15	20	13	20	19
2	25	12	20	13	20	18	20	20
3	20	15	16	12	20	18	20	20
Transmission (per cent)	55.4		71.4		81.7		98.3	
Results from miscellaneous tests	682	404	19	13	28	27	5	5
Transmission (per cent)	59.2		68.4		96.4		100.0	

TABLE 5.—*Incubation period of the new tomato curly top virus in the leafhopper Agalliana ensigera*

Feeding period in the source of virus	Number of tomato seedlings infested and infected[a] by leafhoppers in which the virus had the indicated incubation period in hours:											
	8		16		24		48		72		96	
	Infested	Infected	Infested	Infected	Infested	Infected	Infested	Infected	Infested	Infected	Infested	Infected
(hr.)												
4	12	0	12	0	9	1	12	2	12	2	---	---
8	---	---	---	---	10	2	10	3	10	3	10	1
16	---	---	---	---	22	1	22	3	22	1	10	5
24	---	---	---	---	---	---	10	2	10	5	10	5

[a] Combined data from 4 tests.

a new plant until death of the insect. Fifteen females and 15 males were compared in 2 tests. Results are in Table 6. The new tomato curly top virus was retained in *A. ensigera* for 30 days, and the data suggest that the insects would have retained the virus for longer periods if they had survived longer. Female insects transmitted the virus to 64.2 and 44.8 per cent, and the males to 29.5 and 26.0 per cent of the tomato seedlings in the 2 tests.

Experiments in which viruliferous *Agalliana ensigera* leafhoppers were transferred onto immune sunflower plants, and then tested at intervals, showed that virus was retained by some insects for 46 days. No longer period was tried.

Virus not transmitted by viruliferous parents to their offspring.—Fertilized females of *Agalliana ensigera* which had fed on infected tomato plants for many days were caged on immune sunflower plants for a week and then removed. Half-grown nymphs which hatched from the eggs laid by the viruliferous females on the sunflower plants were tested for virus on young tomato seedlings. Two hundred nymphs were tested in groups of 10 insects per plant on a total of 100 tomato seedlings. In some cases, the insects were left feeding

TABLE 6.—*Transmission of the new tomato curly top to to tomato seedlings on successive daily transfers by Agalliana ensigera*

Test No.	Leafhopper No. and sex	Transmission on indicated successive transfers																												
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
1	1♀	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	−	−	+	+	+	−									
	2	−	−	−	+	+	−	+	−	+	−	−	−	+	−	−	+	+	+	+	+	+	+	+	+	−	−	−	+	−	−	
	3	+	+	+	+	−	+	+	−	+	+	−	+	−	−	+	−	+	+	+	+	+	+	+	+	+	+	+	−	−	+	−
	4	−	+	−	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	−	−	+	+	
	5	+	−	−	+	+	+	+	−	+	−	+	−																			
	6	−	−	+	−	+	+	+	+	+	+	+	+	+	+	[a]	+	+	+	+	+	+	+	+	+	+	−	−	−	+	+	−
	7	+	−	−	+	−	−	−	−	+	+	−	−	+	−	−	+	−	−	−	−	+	−	−								
	8♂	−	+	+	−	−	−	+	−	−	−	−	−	+	−	−																
	9	−	−	−	−	−	−	−	−	−	−	−	−	−	−																	
	10	−	−	−	−	−	+	−	−	+	+	−	−	+	−	−	+	+	+													
	11	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−											
	12	−	+	−	+	−	−	+	−	+	+	−	+	−	+	−	−	+	+	+	−	−	−	+	+	+	−	+	+	−		
	13	−	[a]	+	+	+	−	−	−	+	+	−	+	+	+	−	+	−	+	−	+	−	+	−	−	−	+	−				
	14	+	−	−	−	−	+	+	−	−	+	+	+	+	−	+	−	−	−	−	−	−	−	−	−	+	−	+	−	+	+	−[b]
2	15♀	−	−	−	+																											
	16	−	+	+	−	−	+	−	−	−	−	+	+	−	−	+	+	−														
	17	−	+	+	−	+	−	+	−	+	−	−	+	+	+	−	−															
	18	−	−	−	+	−	−	−	−	+	−	−	−	−	+	−	−	−														
	19	+	+	−	+	−	+	−	−	+	+	+	−																			
	20	−	+	+	+	−	+	+	+	+	−	+	−	−																		
	21	+	+	+	+	+	+	+	+	+	+	−	+	−																		
	22	−	−	−	−	−	−	−	−	−	−	−	−	−																		
	23♂	−	−	−	+	−	+	−	−	+	+	−	+																			
	24	−	+	−	−	−	−	−	−	−	−	−	−																			
	25	−	−	−	−	−	−	−	+																							
	26	+	+	−	−	+																										
	27	−	−	−	+	−	+	−	−	+																						
	28	−	+	−	+	−	−	−	−	−	−	−	−																			
	29	−	−	−	−	−	−	−	+	+	+	−	+	+																		
	30	−	−	−	−	−																										

[a] Plants died from other causes.
[b] This leafhopper was transferred until the 54th transfer, but did not infect any other plant.

on the tomato test plants until they became adult. All results were negative.

DISCUSSION.—Tests have shown that in tomato plantings in the State of São Paulo there is, in addition to the *brasiliensis* variety of sugar beet curly top virus (3), another virus that can also induce curly top in tomatoes. The host range of the new tomato curly top virus is much narrower than that of *brasiliensis*, infecting only a few members of *Solanaceae*, but it also infects *Solanum nigrum* which apparently is not infected by the latter virus. Symptoms induced by the new virus and by *brasiliensis* on host plants susceptible to both viruses are similar. The 2 viruses occur in the same plantings, at the same time, and are transmitted specifically by different species of vectors, the degree of specificity probably being absolute. The incidence of tomato curly top in São Paulo is perhaps the only known instance in which 2 viruses that may be varieties of the same complex occur naturally in the same plantings and have specific vectors.

The persistence of the *brasiliensis* variety of curly top virus in infested areas is easily explained because it can infect a number of other cultivated plants and many common weeds. Spiny bur, a common weed in the State of São Paulo, is very susceptible, and diseased plants are frequently found in natural stands of this plant (3). Infection of tomato plants by the *brasiliensis* variety of virus is probably due to the migration of viruliferous *Agallia albidula* insects from outside into tomato plantings. Transmission from tomato to tomato seems of negligible importance for this type of curly top.

It is not known how the *Agalliana*-transmitted virus is carried over the periods when tomatoes are not planted. Among the weeds tested only *Solanum nigrum* and *Datura stramonium* were found susceptible, and could serve as source of virus for tomato plantings. They are not very abundant, however, and naturally infected plants have not yet been observed. Since the virus does not seem to be seed-transmitted, its persistence in infested areas is most likely to be in the insect vectors or perhaps in some still unknown host plant that acts as a virus reservoir.

It seems likely that the new type of curly top should be easier to eradicate from an infested area than that caused by the *brasiliensis* variety of virus. However, when the new virus is present in a tomato planting its dissemination from tomato to tomato might be rapid because it has a shorter incubation period in the plant than does *brasiliensis*, and its vector, *Agalliana ensigera*, is very efficient in transmitting it from tomato to tomato.

Tests with viruliferous male and female *Agalliana ensigera* transferred daily on a series of healthy tomato seedlings showed that female insects were more efficient vectors than males. These results differ from those obtained by Freitag (6), who had better transmission of sugar beet curly top virus with male than with female *Circulifer tenellus* (Baker). The tests also showed that some insects of either sex are much more efficient vectors than others, suggesting the existence of races that differ in their genetical ability to transmit the virus, as it has been observed for other leafhopper vectors (1, 5, 12).

Differences in host range, the fact that the *Agalliana*-transmitted virus does not infect sugar beet, and its transmission and that of the *brasiliensis* variety of *Ruga verrucosans* by specific insect vectors suggest that the 2 viruses that cause tomato curly top in São Paulo are unrelated. However, variability in the host range of strains of the sugar beet curly top virus has been reported by Giddings (7) who showed that some strains are not able to infect tomato and tobacco plants. The Argentine variety of the sugar beet curly top virus also has a restricted host range (2). Likewise, the existence of different varieties of the same virus transmitted specifically by different species of vectors is already known. Black (4) reported the specific transmission of 2 varieties of the potato yellow dwarf virus (*Aureogenus vastans* (Holmes) Black) by related leafhoppers, and it is known that the sugar beet curly top virus strains that occur in the United States are transmitted by *Circulifer tenellus* which is unable to transmit the variety *distans* of the sugar beet curly top virus of Argentina (2).

The similarity of symptoms induced by the *brasiliensis* variety and by the new *Agalliana*-transmitted virus on the common host plants, and the fact that one vector of this virus, *A. ensigera*, is also a vector of the variety *distans* of the sugar beet curly top virus in Argentina are considered evidence that the *Agalliana*-transmitted virus, present in tomato plantings in São Paulo, is probably a distinct variety of the sugar beet curly top virus complex. The following description for this virus is presented.

PROPOSED NAME AND DESCRIPTION OF THE NEW TOMATO CURLY TOP VIRUS.

Ruga verrucosans Carsner and Bennett var. **solanacearum** n. var.

Virus causing curly top of tomato (*Lycopersicon esculentum* Mill.), characterized by strong vein clearing and curling of leaflets, and stunting of plants. Symptoms remarkably similar to those caused by the *brasiliensis* variety of the complex. Symptoms induced by inoculation of jimson weed (*Datura stramonium* L.), and currant tomato (*L. pimpinellifolium* Mill.) also identical to those caused by *brasiliensis*. It infects *Solanum nigrum* L. which is not susceptible to *brasiliensis*, but does not infect sugar beet (*Beta vulgaris* L.), which is susceptible to *brasiliensis*. Not transmissible through the seeds of infected tomato plants; not transmissible by rubbing, or by needle punctures, to tomato seedlings. Vectors of the virus: *Agalliana ensigera* Oman and *A. sticticollis* Stahl. Virus is not transmitted by *Agallia albidula* Uhl. Incubation period of virus in *Agalliana ensigera*, between 16 and 24 hr. Retained by *A. ensigera* when feeding on immune plants for at least 46 days, but not transmissible from parents to offspring through the eggs. Properties *in vitro* not determined.

Type locality: Campinas, State of São Paulo, Brazil.

SUMMARY

In addition to the *brasiliensis* variety of the sugar beet curly top virus, another virus is present in tomato plantings in the State of São Paulo, causing a type of curly top that cannot be distinguished from the disease caused by the former virus on the basis of symptoms. The relative incidence of the 2 types of tomato curly top varies in different years, but both may be present in the same plantings.

Studies on transmission of the new virus with various leafhoppers and other insects showed that *Agalliana ensigera* and *A. sticticollis* are vectors. Tests with *Agalliana ensigera* and *Agallia albidula*, and the 2 tomato curly top viruses, showed that there is specific transmission of these viruses by their vectors. Negative results were obtained in attempts to break this specificity by needle punctures in the abdomen of the insects.

The host range of the new tomato curly top virus is restricted. Tomato, currant tomato, jimson weed, and *Solanum nigrum* were found susceptible.

The *Agalliana*-transmitted virus was not transmitted to jimson weed by rubbing, nor by any mechanical method to tomato. There was no evidence of transmission through the seeds of diseased plants. The new curly top virus was easier to transmit by grafts than the *brasiliensis* variety.

Agalliana ensigera and *A. sticticollis* fed and bred well on many plants. Sunflower plants, immune to the new virus, were used satisfactorily for rearing these insects in cages. *A. ensigera* fed and bred well on tomato plants, although the same species was reported as being unable to breed on tomatoes in Argentina. *A. sticticollis* did not feed well on tomatoes, and could not be bred on this plant.

Single insect inoculation with *Agalliana ensigera* gave 50 to 60 per cent transmission. with a higher percentage for females than for males. The incubation period of the virus in the vector, *A. ensigera*, was between 16 to 24 hr. The virus was retained by infective insects feeding on immune plants for 46 days, and by insects transferred daily on a series of tomato seedlings for 31 days. The virus was not transmitted from viruliferous parents to their offspring through the eggs.

In spite of differences in host range between the *brasiliensis* variety and the *Agalliana*-transmitted tomato curly top virus, and in the insect vectors of the 2 viruses, the new tomato curly top virus is also considered a variety of the sugar beet curly top virus complex. The name *Ruga verrucosans* Carsner and Bennett var. *solanacearum* is proposed for the new virus.

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A PHYTOPATHOLOGICAL NOTE

Overwintering of Urediospores of Rye Stem Rust in Manitoba.[1] T. JOHNSON AND G. J. GREEN. It has been established that urediospores of *Puccinia graminis* Pers. may survive the winter in the northern United States and Canada to the extent that a small percentage of spores are viable in March, April and even early May. It is doubtful, however, whether any authentic case exists for reestablishment of the rust on new growth by means of overwintered urediospores, except perhaps in timothy rust (*P. graminis phlei-pratensis* (E.&H.) Stak. & Piem.). Therefore it is of interest that a clear-cut instance of overwintering of urediospores followed by an outbreak of uredia on the new growth was observed, at Winnipeg, Man., on couch grass (*Agropyron repens*) in May, 1951.

In a search for teliospores on couch grass on May 1, many life-like uredia were noted on dead but still upstanding stems from the preceding year. Germina-

[1] Contribution No. 1126, Division of Botany and Plant Pathology, Science Service, Dept. of Agriculture, Ottawa, Canada.

tion of the urediospores ranged from 2 per cent in exposed uredia to 20 per cent from stems partially or wholly covered by sheaths. In collections made May 15, germination varied from trace to 17 per cent and, in the last collections, on June 19, from none to 12 per cent. All collections were used for inoculation of wheat, oats, rye and *A. repens* and, in each instance, proved to be rye stem rust (*P. graminis secalis* Eriks. & Henn.).

Observations were made every few days for the appearance of fresh uredia on new growth emerging adjacent to erect rusted stems of the preceding year. New uredia were first found on May 22, when new growth was in the 4 to 6 leaf stage and about 10 in. high. Two pustules were noted, each on a second leaf from the base of a plant. Owing to the location of the pustules, and the fact that they were found about a month prior to the usual appearance of stem rust derived from wind-borne spores, it seems certain that they may be attributed to overwintered urediospores. Cultures from each of these 2 pustules appeared to be pathogenically identical with the cultures previously established from the overwintered spores.

Subsequent spread of the rust was remarkably slow. Although several stems at the infection center became heavily rusted in less than a month, outward spread of the rust could not be detected until June 30, when uredia were found readily within a radius of 3 ft. On July 5, spread of the rust was traced for about twice that distance.

That overwintering also occurred at other points in the vicinity was indicated by the discovery on June 30 of a heavily rusted stem of couch grass at a distance of about 60 yd. from the original infection center. This infection may possibly have arisen from spores dispersed from the first infection center, but the presence of uredia on erect old stems adjacent to the new infection indicated that the rust may also have arisen from overwintered urediospores. On July 11, 3 other infection centers were found, each with about the same amount of rust development as the first one.

The present example is not to be regarded as the first instance of overwintering of rye stem rust in northern areas, though it perhaps represents the first proof that urediospores of this rust may overwinter on dead plant remains and cause infection of new growth. Previous investigations outlined in personal communications from Prof. J. G. Dickson, University of Wisconsin, Madison, Wis., showed that survival of rust occurred in Wisconsin on couch grass and rye in the winter of 1918-1919 by means of the overwintering of mycelium in plant tissues not completely destroyed by freezing.

Conclusions concerning overwintering of rye stem rust should be applied with reservations, if at all, to the economically more important wheat stem rust (*P. graminis tritici* Eriks. & Henn.). In the many studies carried out in past years on the overwintering of stem rust, attention has been centered on wheat and wild barley (*Hordeum jubatum*), the chief grass host of wheat stem rust. Neither Craigie[2] nor Lambert[3] reports any acceptable cases of overwintering of stem rust in cereals in Canada or northern United States, although the latter accepts overwintering of timothy rust. Prof. W. P. Fraser, who for many years studied overwintering of urediospores in Saskatchewan on wheat and *H. jubatum*, was unable to prove any establishment of new infection from overwintered spores, although urediospores, especially those beneath sheaths of *H. jubatum*, frequently proved viable in April and early May. It is only for areas much farther south that records exist of the overwintering of wheat stem rust such as one by Durrell and Lungren[4] who report that in Colorado stem rust did not survive under the dry conditions of the wheat lands but only on wild barley near the edge of lakes and sloughs.

Observations at Winnipeg, in 1951, did not indicate overwintering of wheat stem rust on *H. jubatum* growing among the couch grass on which rye stem rust was observed to overwinter. The uredia on stems and under sheaths of *H. jubatum* looked decidedly less lifelike than those on couch grass, and less than 1 per cent of the spores were germinable on May 1. None germinated from later collections.

Overwintering of the uredial stage in rye stem rust, as in stem rust of timothy, is apparently a normal process of survival in the northern United States and adjacent parts of Canada. The amount of overwintering probably varies from year to year, and there are reasons for supposing that conditions were favorable in 1950-51. Owing to the lateness of spring and summer in 1950, growth of grasses and rust development on them extended far into September. Development of telia was incomplete when cold weather set in; consequently much of the rust was in the uredial stage. Given adequate snow cover, as in the overwintering site at Winnipeg, conditions would be more than usually favorable for the survival of urediospores. Dominion Laboratory of Plant Pathology, Winnipeg, Man., Canada.

[2] Craigie, J. H. Epidemiology of stem rust in Western Canada. Sci. Agr. 25: 285-401. 1945.

[3] Lambert, E. B. The relation of weather to the development of stem rust in the Mississippi Valley. Phytopath. 19: 1-71. 1929.

[4] Durrell, L. W. and E. A. Lungren. Barberry eradication and sources of black stem rust in Colorado. Colorado Bul. 315. 1927.